SCIENTIFIC AGRICULTURE

VOLUME 24 JULY, 1944 No. 11

THE REPRODUCTION OF THE NUCLEUS[1]

STANLEY G. SMITH[2]

Science Service, Dominion Department of Agriculture, Ottawa

[Received for publication March 21, 1944]

INTRODUCTION

Insects have long been and still remain a valuable source of material in the researches of the cytologist. By their study classical contributions have been made to the development of both chromosome cytology and genetics. Moreover, after a period of partial eclipse by plants during the past decade and a half, interest in insect cytology is once more being engendered. In their general uniformity of behaviour plants show the basic principles; insects supply, in their exceptions, the "natural experiments" (Darlington, 7) by which the former may be tested.

It is only within the last few years that the debt to the entomologist is being repaid (see Huxley, 14). It is unfortunate, therefore, that the entomologist is not always prepared to appreciate what he is offered, nor aware of what he can legitimately ask of the cytologist. The reason for this is manifold. Changes in the terminology dealing with chromosome cytology are a natural concomitant of the advances in knowledge that have been made since the majority of us graduated. Unfortunately many of these are confusing either because they resemble pre-existing words or because they are innately difficult to remember. Further, through constant repetition certain hypotheses have not infrequently gained acceptance as established fact (see Huskins, 13); the obvious specialized nature of the study often renders impossible a discrimination between fact and theory except by those constantly concerned with the problems at hand. Finally, there is absent from the literature a description aimed at synthesizing the vast amount of data accumulated from diverse fields without at the same time unduly subjugating them to a preconceived idea. In this outline I have used as much as possible the terminology that is in common use to describe and interpret (often hypothetically) what is seen under the microscope. With the meaning and connotation of the terms the entomologist must become familiar if he is to avail himself of the contributions to the problems of taxonomy and population genetics now being made.

An integration of the type assayed here would be difficult if concerned with so uniform a group as the plant kingdom; to attempt to cover fully so complex a group as the insects, and in a matter of a dozen or so pages, is little short of impossible. We are here concerned only with the funda-

[1] Contribution No. 2307, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada. Based on a paper presented before the Professional Institute of the Civil Service of Canada at Ottawa on February 3, 1944.

[2] Assistant Entomologist; presently stationed at, and Research Fellow in, McGill University, Montreal, Canada.

mentals: the object is to describe the essential facts and, in so far as is possible, to "explain" the cause. Factual exceptions of wide occurrence do not to the best of the writer's knowledge exist; the rare exceptions, because of lack of space, must here be ignored.

No claim is made that the outline presented would meet with the unqualified approval of the majority, nor even perhaps the minority, of cytologists, for, as is usual with the entomologist, the average cytologist is concerned with a particular plant or animal group and can sometimes put his finger on exceptions (real or apparent) in his material that appear to question the validity of the synthesized whole, and of which the synthesizer is unaware. At the same time, it is scarcely conceivable that the micromechanics of such long-established phenomena as mitosis and meiosis could have persisted unaltered in the changing world of biology: evolution is a datum of experience, it must not be expected to circumvent the mechanisms to which its products owe their origin. Even upon such substantial matters as the number of threads in a chromosome there is a singular lack of agreement (Sax and Sax, 22): some say one, some two and others four, and others again just "don't know." Such a divergence of opinion need perhaps occasion no alarm, for we are dealing with objects at the limit of microscopic visibility and, in the case of the "don't know" school, at least, with objects considerably below the powers of resolution of the compound microscope. Then there is the matter of chromosome dynamics: the daughter halves of a chromosome move apart and approach the poles. This much is seen, but how is the movement conditioned? There is no visible fibre connecting the chromosome half and the pole nor, following the results of micro-manipulation experiments, are there invisible *contractile* fibres; hence, there must either be attraction between the daughter chromosome and the pole or repulsion between the daughter halves. The weight of visual evidence favours the latter term for descriptive purposes; electrical potential differences known in living systems are of an order such that the repulsion forces are capable of effecting the movements ascribed to them (Haldane, unpub.).

The method of approach has been deductive-inductive. It is hoped that no undue emphasis has been given to the former, that no pertinent fact has been ignored; it is hoped, moreover, that the end-result will have both heuristic and propaedeutic qualities.

MITOSIS

With few exceptions chromosomes are present in duplicate in the fertilized eggs of insects, one set being derived from one parent, the other set from the other. The similar members of the two sets are said to be *homologous:* they have an ultimate common origin, and affect the same group of reactions in the life of the organism (Sharp, 25). The building up of the body, or *soma*, of the adult involves only about 20 to 30 cell-generations descended from the original single nucleus within the egg (White, 30). As we shall see, accompanying each division, or *mitosis*, of the cell into daughter cells, there is a longitudinal separation of the daughter halves of each chromosome; one daughter chromosome goes into one daughter cell, the sister half into the other. Further, the division of the cell is anticipated by one whole cell generation in the division, or more

correctly, the reproduction of the chromosome[3]. Between each division a *resting stage* intervenes, the active stages of mitosis being the *prophase, prometaphase, metaphase, anaphase* and *telophase.*

Resting[4] *Stage*

When not in an active stage of division, the killed, fixed, and stained nucleus, lying within its *nuclear membrane*, appears to consist of a granular substance within which are embedded two largish, more or less spherical bodies of higher density and refractiveness: these are the *nucleoli.* During the resting stage the nucleoli stain readily, the chromosomes do not[5]. As mitosis proceeds the nucleoli decrease in volume and the chromosomes become increasingly stainable, until, by metaphase, no sign of the nucleoli remains. It is difficult to avoid the conclusion that this is because, during prophase, the nucleoli give up to the chromosomes their stainable substance (though not necessarily chemically unchanged) and become reformed from the chromosomes during the subsequent telophase and resting stage. Accepting this view, the nucleolar material, at one time within the nucleolus and at another within the chromosomes, is always responsive to certain dyes. The transfer of this material from the chromosomes to the nucleolus (and the concomitant cyclical disappearance of the chromosomes) was responsible for the early controversy regarding the continuity of the chromosomes from one division to the next. The belief that the chromosomes are formed *de novo* at each mitosis, like the belief that they are formed by the break-up of a "continuous spireme", has in various ways proved to be groundless.

Prophase

With the exception of the Diptera, the individual chromosomes are, from the very beginning of prophase, always double except at one point, the *centromere*[6] (Figure 1). The centromeres, the dynamic centres of the chromosomes, are grouped, or *polarized*, at one side of the nucleus. The two fine threads, or *chromonemata*, of which each chromosome is composed are, like electric flex, closely paired and twisted together throughout their length, that is, they are *relationally coiled*, and the whole is usually lying in a loose helix (*relic coil*). Apart from linear differences the individual chromosomes are distinguishable only in that two are each in contact at some point along their length with a nucleolus[7].

As prophase proceeds the chromosomes shorten but their total volume increases by taking up the material liberated by the nucleoli: the latter thus gradually diminish in size and finally disappear prior to metaphase. The overall length of the chromonemata in all probability undergoes little if any change, since the apparent shortening is largely due to each assuming the form of a tightly packed helix. This, the *somatic coil*, develops gradually, appearing first as numerous fine undulations, the number of which decreases as their amplitude increases (Figure 2). During this process the relational coil untwists and the larger and looser relic coil is completely

[3] See page 496.

[4] Syn. *energic*: a state of physiological activity without motion of the chromosomes (Berrill and Huskins, 3).

[5] See footnote 14.

[6] Syn attachment region; attachment constriction; insertion region; spindle fibre attachment; kinetochore (Sharp, 24) and kinomere (region) plus kinosome (body) of Huskins (unpub.).

[7] See footnote 14.

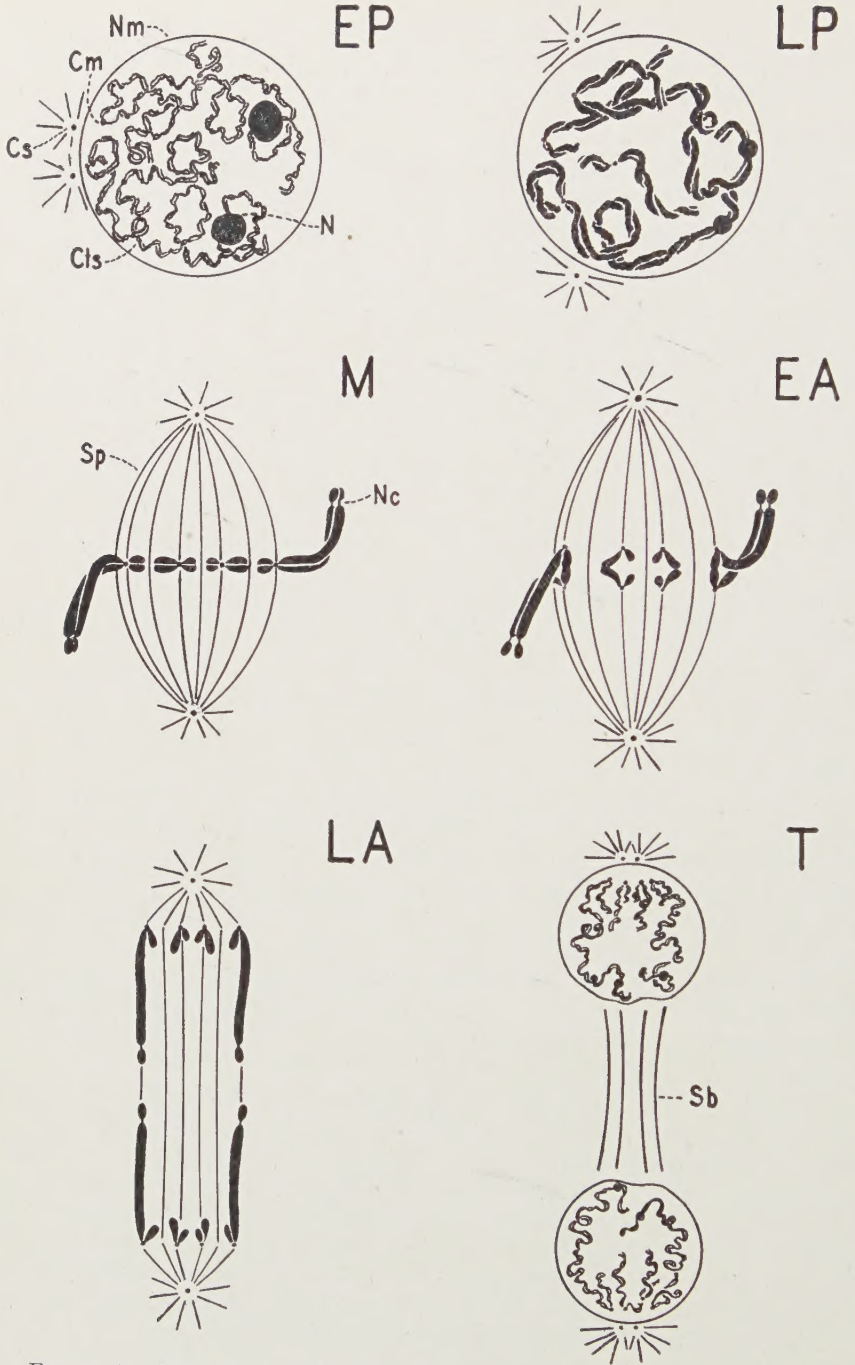


FIGURE 1. The mitotic cycle (excluding the resting stage) in a non-dipterous insect showing early prophase (EP), late prophase (LP), metaphase (M), early anaphase (EA), late anaphase (LA) and telophase (T). Both the relic and the relational coiling of the chromatids are resolved by metaphase. Cm = polarized centromere; Cs = separating daughter centrosomes; Cts = the two chromonemata of a chromosome; N = nucleolus; Nm = nuclear membrane; Sp = spindle; and Sb = stem-body.

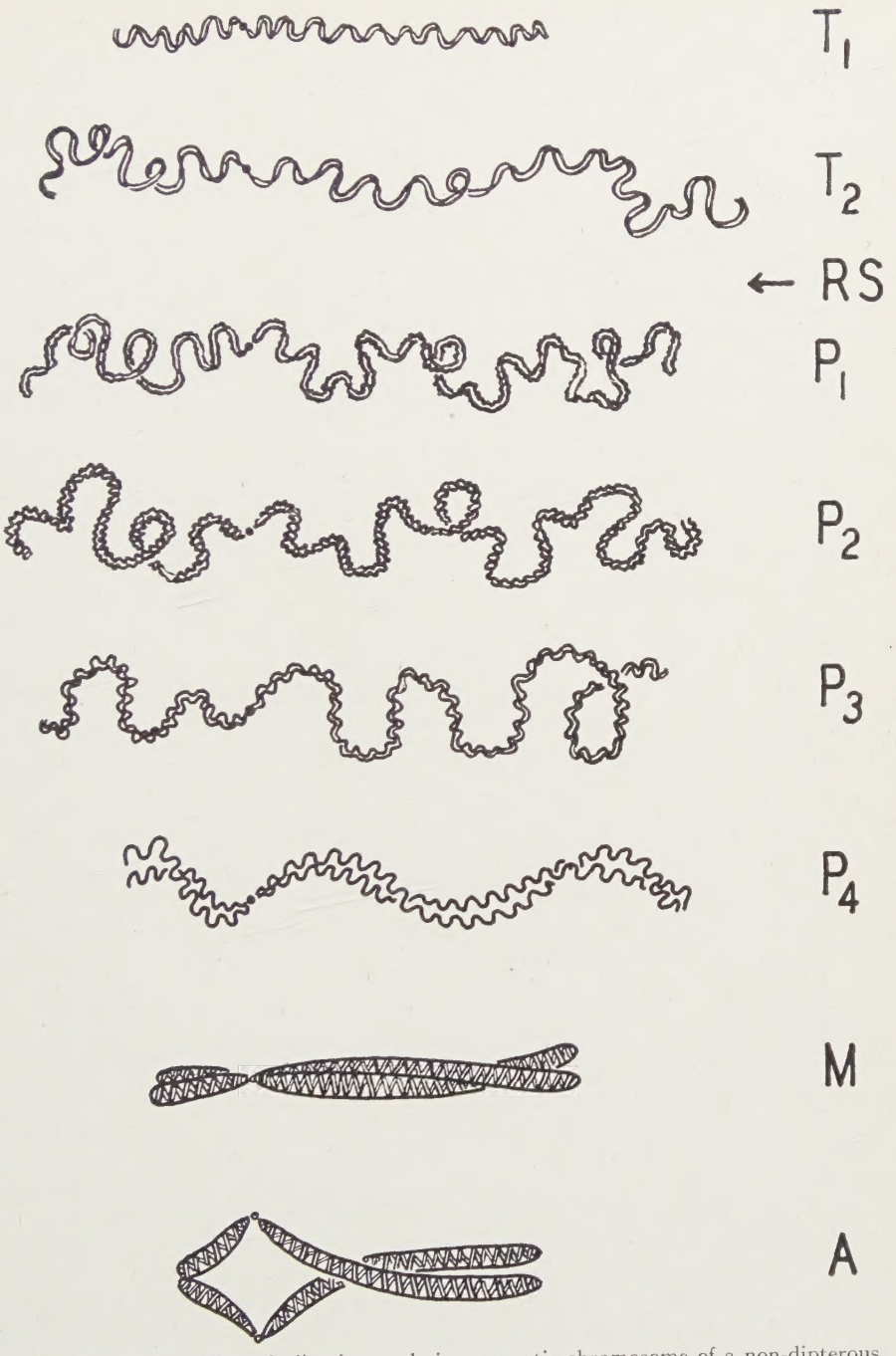


FIGURE 2. The spiralization cycle in a somatic chromosome of a non-dipterous insect starting from the telphase (T_1) of one mitosis and passing through the resting stage (RS unillustrated) to the anaphase (A) of the next. The somatic coils of the bipartite chromosome of T_1 become enlarged and, reappearing as relic coils in the next mitosis, are gradually resolved. At the same time the small undulations appearing in P_1 decrease in number and increase in size to constitute, by metaphase, the somatic coil. The relationally coiled chromatids which reproduce themselves during prophase become visibly bipartite by metaphase.

resolved. The tight packing of the chromonemata into somatic coils together with the nucleolar material accumulated on them causes them to appear by prometaphase as solid, rod-shaped bodies; they are then referred to as *chromatids*. Their true internal structure is usually demonstrable only following special methods of treatment.

Prometaphase

At the end of prophase, or by *prometaphase* as it is called, the chromosomes have reached their maximum contraction, the nuclear membrane disappears and the process of *spindle formation* occurs. This is a variable process but typically may be described as occurring in the following manner. As early as prophase, or even earlier, the two *centrosomes* lying close together and just outside the nuclear membrane start to move apart[8]. Having reached opposite sides of the nucleus by prometaphase, the centrosomes set up two poles and the nuclear membrane breaks down thus allowing the invasion of the nucleus by the contents of the cytoplasm. Under the action of the centrosomes the nucleo-cytoplasmic particles become orientated end-to-end and the aggregates so formed finally reach from pole to pole. These so-called fibres contain between them the more fluid portions of the nuclear sap. These more fluid interstices constitute "skids" along which the repulsion forces of the centromeres are able to act more freely. The chromosomes (paired chromatids) appear to be endowed also with a more general force of repulsion for they are dispersed more or less evenly throughout the cavity of the late prophase nucleus; maximum dispersion is reached by prometaphase. The mutually repellent forces of the chromosomes and especially of their single centromeres, on the one hand, and the two centrosomes, on the other, force the centromeres of the chromosomes to *congress*, or lie collectively, at metaphase, in an equatorial plate midway between the two poles. The centromeres then become associated with some of the fibres, which are then known as half-spindle components, while those uninterrupted by centromeres remain as continuous, or *primary*, fibres extending from pole to pole.

Metaphase

If the chromosomes are short, as in the Lepidoptera, the whole body of each chromosome of necessity lies flat on the metaphase plate. If long, as are some in the Orthoptera, they lie with their *distal* parts, i.e., the ends away from the centromere, projecting either out from the periphery, floating freely in the surrounding cytoplasm, or into the half-spindle masses on either side of the plate. As a general rule, where both large and small chromosomes occur together, as in the Heteroptera, the former assume a peripheral position, and the latter lie in the middle. Apart from this the members of the complement lie at random on the plate (except in the Diptera—see later). All these positions seem to point to a generalized repulsion keeping the chromosomes at a maximum distance from one another.

[8] The centriole (≡ centrosome) in viviparous snails has recently been shown by Pollister and Pollister (21) to be equivalent to a centromere, differing in behaviour in being isolated in the cytoplasm, while the latter lies within the nucleus as part of the chromosome. Moreover, it appears probable that centriole reproduction is from material derived from the centromeres which flows out from them along the half-spindle components. Hence the time of centriole reproduction seems to be limited to the time during which the spindle has its existence.

That the centromere is the main factor in the arrangement and movement of chromosomes is shown by the behaviour of chromosome fragments lacking a centromere (*acentric*) and other abnormal chromosomes possessing two (*dicentric*). The former fail both in normal congression and in normal anaphase movement[9]; the latter fail (in about half the cases) only in the anaphase separation of their chromatids, for the two centromeres of a daughter chromosome may as often move towards opposite poles as towards the same pole.

The position of the centromere is constant for each individual chromosome but may be different in different non-homologous chromosomes in the set. Thus chromosomes may be described as *mediocentric*, *telocentric* or *subtelocentric*. The position of the nucleolar constriction is invariable. It develops as a result of the inability of the chromosome to spiralize in that region where it was embedded in the nucleolus during prophase.

Only in the symmetrical field set up by the chromosomes congressing at the equator can the centromeres divide; this they do longitudinally by the time that the two chromatids have completed their reproduction of daughter chromatids. Thus in division the centromere is one whole cycle behind the chromosomes and almost one whole cycle behind the centrosome.

We have seen that (1) during the prophase the contents of the nucleolus have been distributed among the chromosomes; (2) during this distribution the chromosomes become progressively more stainable; and (3) with its completion the chromatids of each chromosome become visibly double. This splitting or division of the chromatids, as it is loosely called, involves not the longitudinal cleavage of the chromatid but the attraction and orientation by its particles of similar ones, so that one thread reproduces and becomes two. It is as though the nucleolus is the site for the storage during the resting stage of the excess material not used in the previous chromatid reproduction, together with additional material synthesized from the products of cellular metabolism. This material might then become transferred to and orientated by the molecules of the chromatids during the succeeding prophase, culminating in the reproduction of the daughter chromatids by metaphase. Hence, it seems, reproduction might be dependent on the association of nucleolar material with the chromatid thread. This is uncertain, but if true, reproduction cannot occur at the resting stage (*contra* Darlington, 9) but must have been occurring during the prophase of the previous mitosis[10].

Anaphase

The dual effect of the two new daughter chromatids, lying together and thus strongly repelling each other, together with the pairs of paired half-chromatids, releasing the attraction between single chromatids[11], moves the daughter chromosomes apart. The centromeres take the lead

[9] See footnote 11.

[10] Some authors are of the opinion that the chromosome at anaphase consists of one thread; others consider there are two or more. Reproduction according to the former authors occurs during the resting stage (since the chromosome is visibly double at the earliest prophase); opinions in the case of the latter group are variable. The unified theory of the writer is based on the chromatid as a single body and as the functional unit in pairing, chiasma formation and crossing-over, and in its mutational response to X-radiation.

[11] Carlson (4) has shown that daughter chromosome fragments devoid of centromeres and lying outside the spindle, though in the equatorial plane, can separate simultaneously with normal, unfragmented chromosomes; they lag, however, in later movement.

in this movement[12] so that a mediocentric daughter chromosome assumes the form of a V, with the apex pointed towards the pole, and a telocentric one the form of a rod, with the end at which the centromere is situated similarly pointed towards the pole. Where long and short chromosomes occur together, the daughter halves of the latter will be separated well in advance of those of the former; in the case of submediocentric chromosomes, the shorter arms will be separated before the longer arms.

The movement towards the poles is facilitated by the waning of the centrosome repulsions, perhaps due to a change in the nature of the substrate that adversely affects their repulsion, but more likely due to the centrosomes themselves undergoing reproduction in preparation for the following mitosis[13]. When the centromeres have travelled about two-thirds of the distance between the equator and the poles, autonomous movement ceases, and further movement is effected by the elongation of the middle region of the spindle lying between the two groups of separating centromeres (Belar, 1). This middle region, or *stem-body* as it is called, not only moves the two groups of daughter chromosomes apart, but the two poles also, so that the overall length of the spindle is greater at the end of anaphase than it was when originally formed.

Telophase

Due to the elongation of the spindle the two groups of chromosomes never quite reach the poles and consequently the centromeres in each group never become as closely aggregated as they would were this position attained. With the completion of the polar movement a new nuclear membrane is formed around each of the telophase groups and an incipient nucleolus appears. The chromosomes (paired chromatids) undergo initial "*de-spiralization*" and become progressively less stainable as they pass into the resting stage. The loss in stainability is completed by the resting stage[14]. The process of de-spiralization, however, is halted only temporarily: it restarts in the following prophase and is completed by mid-prophase. It consists not in a reversal or undoing of the spirals but, rather, in a continuation of the coiling which had its inception in the previous prophase. The spirals, at first large in number and small in diameter, gradually decrease in number and increase in size. At telophase this expansion is only about half completed, but by the following mid-prophase the number is reduced to a minimum and the size is increased to a maximum—hence the term relic coil.

There is no movement during the resting stage. At prophase the chromosomes are orientated, as they were at anaphase, with their centromeres against one side of the nuclear membrane and their arms radiating out towards the opposite side: they are polarized.

From this general account it will be seen that mitosis consists of a series of cyclical, co-ordinated changes and movements, both within and without the chromosomes, jointly concerned with the orderly separation of

[12] The centromeres at meiosis in *Sciara* (Metz, 19) and *Micromalthus* (Scott, 23) are exceptional in this regard.

[13] See footnote 8.

[14] Loss of stainability involves the return to the nucleolus of material earlier accumulated from it; generally, parts of the chromosomes or even whole chromosomes retain this material which then continues to stain deeply in the resting stage. Regions which show the normal cyclical staining properties are called *euchromatic*, those which do not are *heterochromatic* or *heteropycnotic*; the latter were originally known as *prochromosomes* and are often, if not always, genetically inert.

the daughter chromosomes. These involve (1) a lengthwise contraction of the chromosome by the development of a somatic coil, which appears to serve no end other than a convenience in mobility at metaphase congression and at anaphase separation; (2) the reproduction by metaphase of half-chromatids, attraction between which replaces the earlier attraction holding chromatids together; and (3) a co-ordination of the components of the substrate as an aid in the movement apart of the daughter chromosomes.

Somatic Pairing

The somatic chromosomes of the Diptera differ from those of all other insects, and, in fact, all other known organisms, in that throughout every mitosis the members of homologous pairs lie in close association with one another (Figure 3). Because of this there may appear to be only the *haploid* (single) number of chromosomes present rather than the *diploid* (double) number. The mechanical basis for this paired condition in the Diptera lies, at least in so far as can be seen, in that the chromosomes are single at anaphase[15]. The "division" of the body of the chromosome into daughter chromosomes and the centromere into daughter centromeres is synchronized, both being completed at the metaphase immediately preceding the anaphase in which daughters are to separate. At prophase the pairs of relationally coiled chromosomes differ in appearance from the pairs of relationally coiled chromatids in other forms only in that each has two centromeres, one the property of one chromosome, the other that of its homologue. By metaphase the relational coiling is completely resolved; also by this time each chromosome including the centromere has become double. The resolution of the relational coiling and the reproduction of the chromosomes allows the arms to separate, but the centric regions remain close together because of their anchorage to neighbouring half-spindle components. The derived centromeres of each chromosome then separate, moving daughter chromosomes to opposite poles, each closely paralleled by a daughter chromosome from a homologue.

So much for the manner in which the paired condition is maintained, but how does it originate? When the single set of chromsomes introduced by the male at fertilization lines up with that of the egg on the spindle of the first cleavage division, each chromosome of the double set has reproduced its arms and its centromere, and the daughter chromosomes separate to constitute the anaphase of this division. By virtue of the singleness of each anaphase chromosome, those of homologous pairs attract one another and re-establish the paired condition that was temporarily existing at metaphase (unpublished data). The paired chromosomes, lying closely parallel by telophase, undergo the resolution of their somatic coils and at the same time twist around one another (cf. Kaufmann, 15, Figure 56) to reappear at the succeeding prophase as relationally coiled pairs.

This phenomenon of somatic pairing may, in some Diptera, extend even into *meiosis*, i.e., those mitoses especially modified for the production of germ cells, and it is in all probability directly responsible for the occurrence of the giant, so-called *salivary gland chromosomes* peculiar to the Diptera.

[15] It is generally believed (following Kaufmann, 15) that the chromosomes of the Diptera are, like those of all other known organisms, double at prophase: their association in homologous pairs is, therefore, attributed to "a specially exaggerated property of attraction" (Darlington, 9). There is no unequivocal evidence for their doubleness and hence no demand for rationalization (Smith, 27).

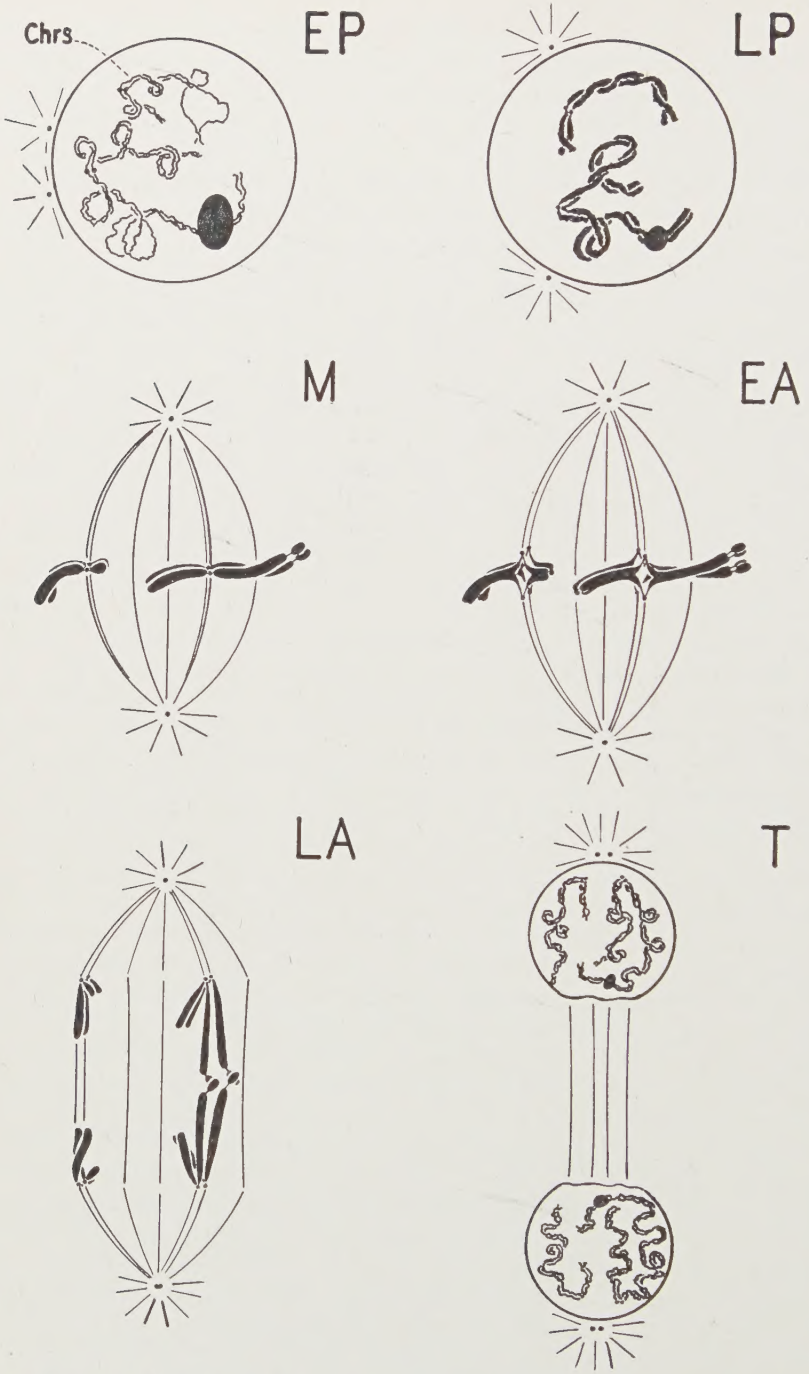


FIGURE 3. Mitosis in the Diptera: same stages as in Figure 1. At earliest prophase the chromosomes (Chrs) are single but homologous pairs are relationally coiled. By metaphase both reproduction of the chromosome and unwinding of the relational coil are completed. At anaphase single daughter chromosomes separate and homologues associate in pairs. They again become relationally coiled at telophase.

Salivary Gland Chromosomes

Within the nuclei of the secretory glands of the Diptera the chromosomes appear as enormously enlarged multiple threads present in the haploid number. They are almost certainly formed by the repeated reproduction of the paired (*contra* Koller, 17) chromosomes originally associated in the phenomenon of somatic pairing. The multiple threads, or *polytene* chromosomes, are transversely striated with bands of varying thickness which stain readily with ordinary nuclear dyes and which are separated by non-staining bands also of varying thickness. The polytene chromosomes are often fused together at their centromeres to form the *chromocentre* from which the nucleolus is suspended by a fine thread. Each band is actually a disc extending through the thickness of the polytene chromosome and made up of a number of granules, or *chromomeres*, each of which is joined by a fine filament to a granule in the disc on either side. Even in regions where the bundle of threads is separated into its two component groups, derived from the two homologous chromosomes, the similarity of the bands in the two is always exact, not only in a cell, but, apart from mutation, in all cells of individuals of the same species.

It would appear that these polytone chromosomes have arisen through the repeated reproduction of the somatically paired chromosomes unaccompanied by the compensating division of the cell[16], [17]: factors contributing to their remaining together are (1) the spindle has failed to form; (2) their prophase relational coiling has failed to become resolved; and (3) attraction in pairs is probably localised, exchanges of partners being possible as a result of the persistent relational coiling.

MEIOSIS

Meiosis and fertilization are antitheses. Fertilization consists in the union of two *gametes*, or germ-cells, in forming the *zygote*; meiosis consists in two divisions of the nucleus of the zygote accompanied by one division of its chromosomes in forming the gamete. Thus meiosis results in the chromosomes being numerically reduced to half the diploid number; fertilization results in the restoration of the double number.

Meiosis, then, is accomplished by two divisions of the nucleus, but the most distinguishing characteristics which differentiate it from mitosis are confined to the first of these divisions. The special characteristics of the process result solely from the chromosomes being single at the onset of the first division: they are not divided into chromatids. Their singleness arises from an anomaly in the mitosis immediately preceding meiosis, that is, in the last premeiotic division of the spermatogonia and oogonia[18]: the body of the chromosome, which throughout the earlier mitoses, has preceded the centromere in reproduction by a whole division cycle, at last loses this priority so that both are reproduced at the same time: synchrony in reproduction is achieved and, as we have seen in the Diptera, the conditions

[16] Goldschmidt and Kodani (10) have unorthodox views regarding the structure of polytene chromosomes.

[17] It should be noted that if chromosome reproduction entails the transfer of the contents of the nucleolus to the chromosomes, then there should be a rhythmic disappearance of the nucleolus synchronized with each reproduction. There is unfortunately no evidence on this point.

[18] Darlington, who considers the chromosome always to be single at anaphase with reproduction occurring at the resting stage, believes that meiotic prophase is precocious, occurring before the chromosomes have reproduced themselves: on this he bases his precocity theory of meiosis (Darlington, 6).

necessary for somatic pairing are established. When the daughter centromeres separate at anaphase, they disjoin not paired chromatids but single daughter chromosomes. The daughter chromosomes are thus in a condition strictly comparable to the daughter chromosomes at the anaphase of the first cleavage division in dipterous eggs. Moreover, their immediate reaction is comparable: homologous pairs are mutually attractive, an association is established, necessarily loose because of their spiral form, first in the centric regions and later sometimes in more distal regions, and, by unwinding their somatic coils, they proceed to coil relationally. In this state of greater or lesser association they pass into the resting stage.

The condition of the chromosomes at the earliest prophase of the first meiotic division is dependent upon the degree to which both the pairing and the relational coiling had progressed before the immobilization of the resting stage sets in. When first they become visible, at *leptotene*, they appear as long, thin, contorted, faintly-staining threads upon which are imposed numerous heavily-staining, bead-like chromomeres of various sizes (Figure 4). Although, on account of their length, it is rarely if ever directly demonstrable, these leptotene chromosomes are usually polarized with their centromeres towards the pole: this follows from their prior telophase orientation and the immobility of the resting stage. Each chromosome, *per se*, is single [19] but each is spatially in more or less close association with its homologue. This loose, *en masse*, pairing is then replaced by an intimate chromomere by chromomere pairing. This is the *synapsis*[20] of *zygotene*[21]. It commences primarily at the centromeres, since they lie closest—though it may be taken up secondarily by other homologous parts which also chance to lie closely enough together—and progresses outwards along the arms (Smith and Boothroyd, 28). With its completion the diploid complement of chromosomes is present as the haploid number of *bivalents*.

It is only now, at *pachytene* as it is called, that the reproduction of the chromosomes, omitted at the preceding metaphase, occurs; the object in this omission has been accomplished, the chromosomes are in pairs. As each chromosome of a bivalent becomes double, the attraction between its

[19] On the basis of the observable doubleness of the chromosomes at mitotic anaphase and telophase, some authors are loath to consider leptotene chromosomes as *single* pairing units; their direct evidence for doubleness at leptotene is questionable or absent. It is possible, however, that heterochromatic regions may be double.

[20] The anaphase-telophase of the last premeiotic division is thus the time of *conjugation* (as opposed to chromomere synapsis): here the chromosomes act as units in the same sense that *Paramoecia* are said to conjugate.

[21] Where doubleness is developed locally prior to zygotene no synapsis follows (Huskins and Smith, 11).

FIGURE 4. Meiosis in an insect that shows genetic crossing-over. Leptotene (L) showing single homologues with relic coils loosely associated in pairs and relationally coiled. Zygotene (Z) showing chromomere by chromomere pairing. Pachytene (P) showing pairing almost completed: the pairs are now 4-partite except at the centromeres. At early diplotene (ED) one chiasma (Xma) is visible in the shorter bivalent and three in the longer one; association otherwise is restricted to pairs of chromatids. During mid-diplotene (MD) the chiasmata move away from the centromeres and internal coiling is initiated. Diakinesis (Dia) showing complete terminalization of two of the chiasmata; maximum contraction is now reached. The first metaphase (MI) involves coorientation of the centromeres and anaphase (AI) shows the separation of the "homologous" chromosomes. Second metaphase (MII) and anaphase (AII) shows the orientation and separation of the internally double daughter chromosomes.

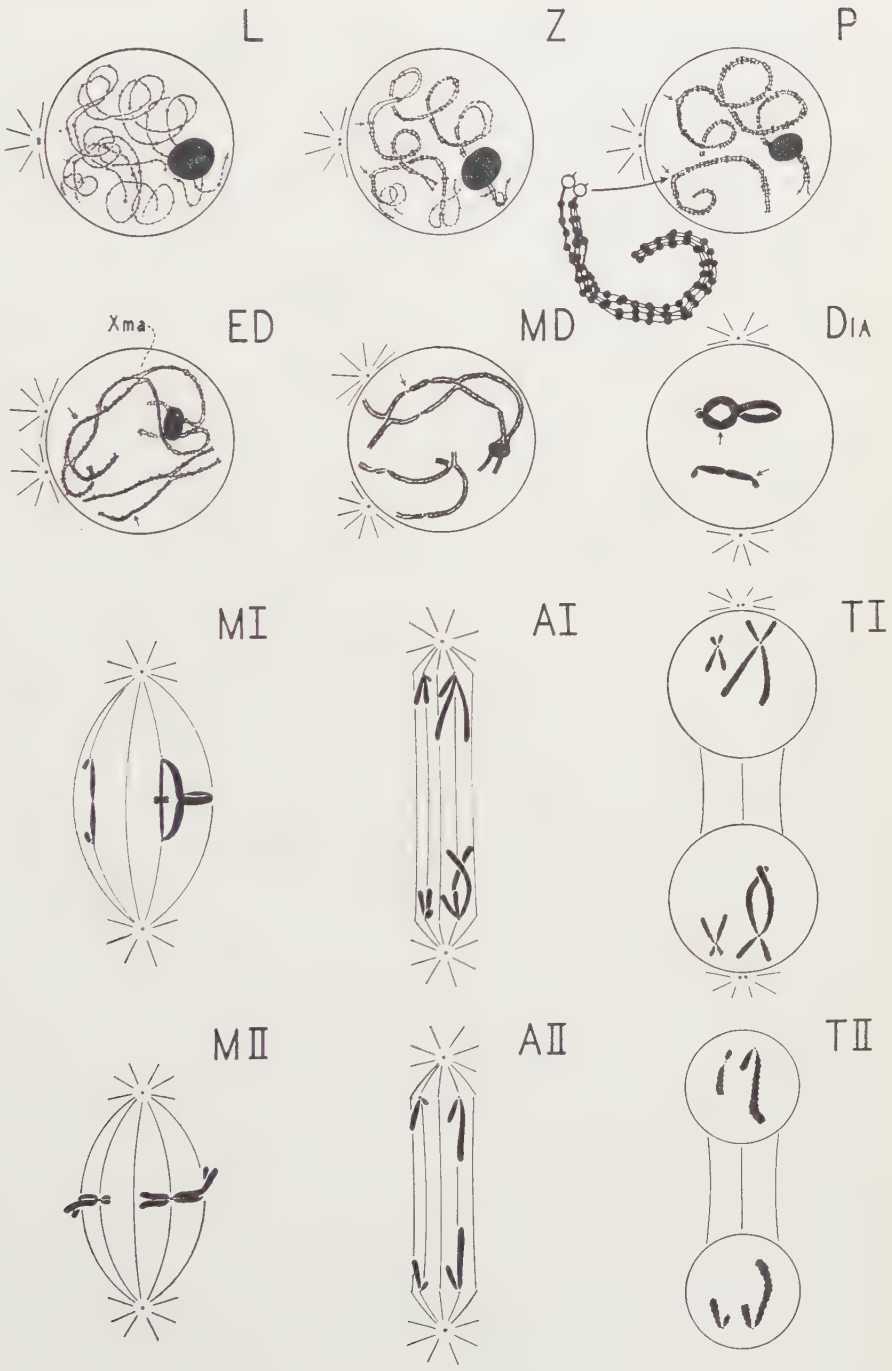


FIG. 4.

chromosomes lapses, for attraction is limited to pairs. The pairs of paired chromatids cannot separate completely, however, for it is seen that reciprocal exchanges have occurred between one thread of each pair; the two threads involved cross each other to form a *chiasma*. It is known that on either side of the chiasma those chromatids actually in paired association are the same ones as those originally formed at the delayed reproduction just mentioned (Darlington, 5). Hence the chiasma must be formed either by physical breakage of two homologous (not sister) chromatids followed by the criss-cross reunion of their broken ends (Darlington, 8), or by connecting fibres joining chromomeres of homologues at the reproduction of the chromatids at pachytene (Belling, 2). Which of these hypotheses, if either, is correct is unknown[22], but the chiasma, the end-result, is visible—it is the cytological manifestation of genetic crossing-over.

The pairs of paired chromatids move apart except, of course, where linked together by chiasmata. At this stage, *diplotene*, the bivalents, or *tetrads* as they are commonly called, if associated by one chiasma show the form of a cross, if by two, the region between them assumes the form of a circle.

As the diplotene stage progresses the chromosomes undergo spiralization, becoming broader and shorter, until, at *diakinesis*, they reach their maximum contraction and, because a general repulsion is still operative as at mitosis, they lie against the containing nuclear membrane more or less evenly dispersed; the nucleolus has already disappeared, the membrane dissolves, and the spindle of the first meiotic metaphase forms.

In the meantime certain changes of greater or lesser magnitude may have occurred in the disposition of the chiasmata. If the chromosomes are large, as are some in the Orthoptera, the metaphase positions of the chiasmata will be little or no different from that at their time of formation. If they are small, as is more generally the case with insects, the chiasmata *terminalize*, i.e., the crosses representing the genetic cross-overs move, by changes in association on either side, away from the centromeres[23]. If this terminalization is complete crosses become rods and tetrads with one or more chiasmata in each arm become simple rings[24]. In intermediate cases, where terminalization is incomplete, there may be only a shifting towards the ends, or where more than one chiasma is present in an arm, only the most distal chiasma may move to the end, the other or others moving a little but remaining interstitial. Whatever its degree of completeness, the association of the two segments containing the centromeres extends at the expense of the neighbouring segments not containing them: it is clear that terminalization is an aid in the separation to follow at anaphase.

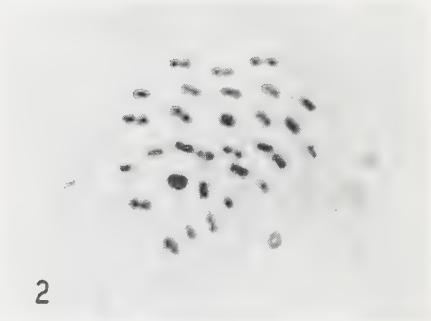
The congression of the chromosomes on the equatorial plate at the first metaphase differs from that of mitotic chromosomes at the comparable stage. In the latter the centromeres lie in one plane on the equator and then divide. In meiosis the two pairs of chromatids composing the tetrad are each provided with a centromere and these are single and remain so until the metaphase of the second division. Instead of each lying on the

[22] Darlington (8) postulates torsion developed from a molecular coil as responsible for breakage.

[23] Mather (18) is of the opinion that terminalization is an all-or-none process.

[24] According to some authors a matrix (of extra-chromatic material?) is responsible for holding the chromosomes together following complete terminalization and in other instances where no visible connection is apparent.







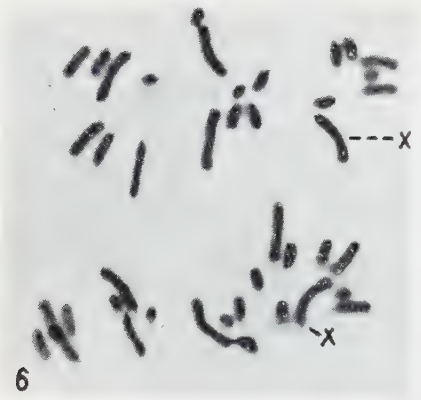




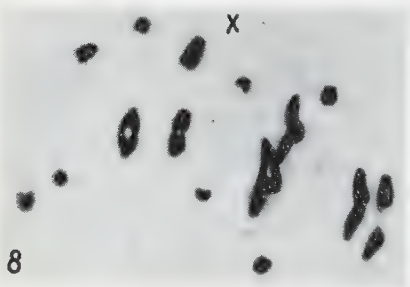
EXPLANATION OF PLATES I-III

Photomicrographs taken from slides prepared by the squash method (Modified Kahle's fluid and leuco-basic fuchsin, Smith, 29).

PLATE I, 1-4. First metaphase of meiosis in male Lepidoptera. 1, *Zale* sp., 31 bivalents. 2, *Archips cerasivorana*, 30 bivalents. 3, *Archips* (*Cacoecia*) *fumiferana* from jackpine, 30 bivalents. 4, *A. fumiferana* from balsam, 30 bivalents. 5, First anaphase of meiosis in male *A. fumiferana* from balsam. Magnification = 2000.









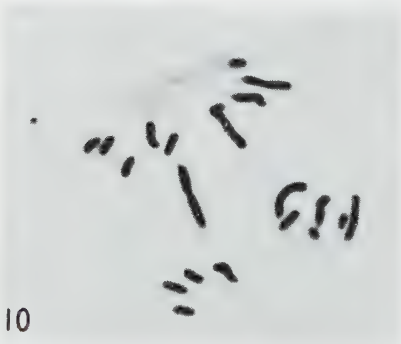
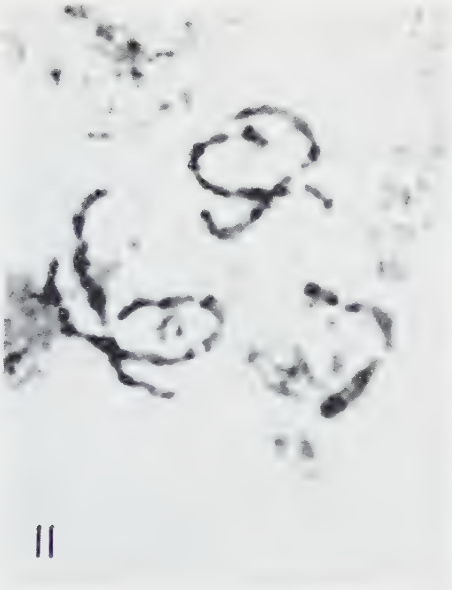


PLATE II, 6-10. Mitosis and meiosis in male of *Zubovskya* (*Podisma*) *glacialis canadensis* Walker (Acridiidae). 6, Spermatogonial mid-anaphase showing chromosomes conjugating in pairs prior to meiosis. 7, Pachytene showing the condensed X chromosome paired back on itself and heteropycnotic knobs on one end of each of the ten bivalents. 8, First metaphase of meiosis; ten bivalents plus the unpaired X chromosome. 9, First anaphase; five of the smaller bivalents have disjoined and the X chromosome has moved towards one pole. 10, Second anaphase in an X-containing daughter nucleus; the X chromosome has yet to complete division. N.B. the chromosomes in this species have terminal centromeres. Magnification = 1400.



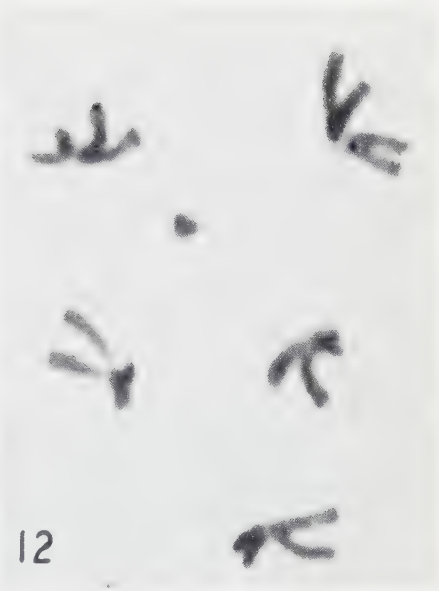






PLATE III, 11-14. Mitosis and meiosis in males of *Phorocera hamata* (Diptera). 11, Mitotic prophase showing somatic pairing; five "bivalents" plus a small XY pair. 12, Mitotic metaphase with non-coorientated centromeres. 13, Anaphase showing parallel association of daughter homologous chromosomes. 14, First metaphase of meiosis; the centromeres of the bivalents are coorientated, hence the XY pair looks single (the coorientation of one bivalent has been upset in squashing: it therefore resembles a mitotic pair). Magnification for 11, 12, 13 = 2000; for 14 = 3200.

equator, they arrange themselves symmetrically, one on either side of the equator. Ideally, then, it is the proximal chiasma that lies on the equator: the centromeres repel each other (as they did in terminalization) and become axially *co-orientated*.

Anaphase does not await the reproduction of the chromatids as it does in mitosis: there is no attraction to be overcome, the tetrad is already four-partite, a system of pairs repelling pairs. *Disjunction* commences with the attenuation of the regions between the centromere and the nearest chiasma, and the greater the number of unterminalized chiasmata, the greater the stretching. Finally, however, the pairs of paired chromatids separate, the two associated by a common centromere going to one pole, the other two to the second.

The dyads pass into the telophase which is followed by a very short resting stage, or *interphase* as it is called, and reappear in much the same condition at the prophase of the second division. Cytologically this division is mitotic in nature: as we shall see below, genetically it ensures the purity of the gametes. During prophase the centromeres and the body of the chromosome reproduce themselves, the chromosomes congress, and the daughter chromosomes (again internally double except in the Diptera) separate to form the nuclei of the gametes.

In those organisms in which there is no interphase and in which there is, therefore, no nucleolar-formation, the reproduction, above stated to occur during the second prophase, is accomplished between pachytene and metaphase of the first division. The chromosomes at the first anaphase are thus four-partite and arrive on the second division plate with no further reproduction.

The first important consequence of these two divisions is that the diploid number has been reduced to the haploid number which, owing to segregation, includes one member of each pair. Second, because the orientation of the pairs of centromeres is independent for each pair of chromosomes, random assortment of the chromosomes of each set has taken place. Third, these assorted chromosomes have undergone an alteration from their premeiotic constitution: homologous ones have exchanged parts, crossing-over has occurred. Now we see why the second division of meiosis is necessary: the numerical reduction is accomplished in the first division but the dyads are constituted of regionally dissimilar chromatids: they must themselves segregate at the second division. Hence we get four nuclei all different from one another and none exactly like either the paternal or the maternal half of the complement in the mother-cell. Meiosis thus introduces the maximum recombination of genetic differences. In the male the four nuclei all form sperm; in the female three are cast out of the egg and the fourth alone functions as the female pronucleus.

Fortuitous Univalents and Sex Chromosomes

Chiasmata are in general the prerequisites to an orderly chromosome reduction. In their absence, due either to mechanical difficulty in pairing or to chance failure, a bivalent will be represented by two *univalents*, i.e., the pair of chromosomes are *asynaptic*. The univalents may divide at either the first or the second anaphase or very infrequently at both, but

their products often fail to reach the telophase nuclei. Changes in chromosome number, either plus or minus, result, which, however, will rarely persist in future generations.

XO Sex Determination

Gametes with different chromosome numbers are, however, the normal products of meiosis in certain species of insects. These are those, like the majority of Orthoptera, in which the sex chromosomes are present twice in one sex (the female) and only once in the other. The non-sex chromosomes, or *autosomes*, are of course present in both sexes in duplicate. In the Orthoptera the single X of the male goes undivided (by an unknown mechanism) to one pole and its daughter halves disjoin at the second anaphase: two types of sperm in equal numbers are produced, the one with, the other without the X. In the female, on the other hand, the two X chromosomes behave as do the autosomes and all eggs come to possess the X chromosome. These may be fertilized by X sperm or by no-X sperm: the former give rise to female, the latter to male zygotes. The alternative condition in which the single X divides at the first anaphase and goes undivided to one pole at the second is the general rule in males of the Coreidae (Heteroptera): the end-result is identical.

Haplo-diploid Sex Determination

The extreme of the XO type of sex determination is met with in facultatively parthenogenetic Hymenoptera where males develop from unfertilized eggs and females from fertilized ones; the male is therefore haploid, the female diploid. All the chromosomes are, in a sense, sex chromosomes[25]. Meiosis in the female is again normal, but in the male, by virtue of the absence of homologues, pairing is impossible: all the chromosomes fail to divide at the first anaphase (although they have completed reproduction); instead they all move to one pole and the second division is purely mitotic. The mechanism governing this regular movement to one pole is unknown. All sperm produced by an individual therefore have the haploid number of chromosomes and all have the same genetic constitution. Prior to fertilization all eggs likewise have the haploid number of chromosomes—because normal meiotic reduction has been accomplished, but these may be genetically different—because crossing-over has occurred. If fertilized they will give rise to females, if unfertilized to males[26].

XY Sex Determination

The departure from normal meiosis is, with the exception of many Heteroptera, here less radical in that the behaviour of the sex chromosomes is fundamentally similar in both sexes to that of the autosomes. The X

[25] Whiting and his associates (see Whiting, 31) have clear evidence of multiple sex genes in the parasitic hymenopteran, *Habrobracon juglandis*: haplo-diploidy is therefore a secondary mechanism in this insect.

[26] The failure to form bivalents in the male hymenopteran would at first sight appear to be due to the absence of a second set of chromosomes. We find, however, that in those rare clusters of diploid spermatocytes that have arisen as the result of an early spermatogonial irregularity bivalents are likewise not formed (Smith, 26). There is no evidence why this is so, but it is conceivable that in haploid males there is no suppression of the last premeiotic reproduction which according to theory (Smith, 27) is essential to meiotic pairing. In the absence of homologues such a prerequisite to pairing would lose its significance; there would be no selection against a reversal to purely mitotic reproduction.

chromosome is present in duplicate in one sex; in the other it is accompanied by a chromosome, the so-called Y, which may differ from it morphologically as well as genetically[27]. With the exception of the Lepidoptera[28] and Trichoptera (Klingstedt, 16), it is the male insect which is *heterogametic*, i.e., capable of producing two types of gametes as regards the sex chromosomes. In these two orders the male is *homogametic*; it produces only X-containing sperm. In many pentatomids (Heteroptera) the X and Y chromosomes divide independently at the first division but come together at the second metaphase and then separate without further division: the end-result is similar in giving X and Y sperm in equal numbers.

Included in those forms having XY males is the Diptera. Dipterous males fall into two categories: those in which chiasmata are formed, as for example the Culicidae, Chironomidae, etc.; and those comprising all the higher forms (Brachycera) in which chiasmata and genetic crossing-over are absent. All show the phenomenon of somatic pairing and all females, but males of only the former type, have normal meiosis. Here the special timing lag by which reproduction and division occur together and which determines somatic pairing breaks down at the prophase of the first division of meiosis. The chromosomes, entering meiosis in the paired condition, complete their reproduction during the early prophase rather than at metaphase: crossing-over occurs and chiasmata hold the pairs of paired chromatids together until anaphase. In male Brachycera the reproduction-division cycle is undisturbed. The somatically paired chromosomes condense and spiralize before reproduction is completed and hence prohibit chiasma-formation. In one respect, however, the first anaphase separation in the Brachycera resembles that of other Diptera, rather than that seen in an ordinary dipterous mitosis. Separation is completely *reductional*, i.e., a whole paternal chromosome separates from a whole maternal homologue, whereas in mitosis the separation, as we have seen, is *equational*, i.e., one paternal and one maternal chromatid separate from a second paternal and a second maternal chromatid. Apparently at meiosis, whether chiasma-formation occurs or not, the spindle becomes operative before the centromeres can congress and divide: instead they co-orientate and disjoin.

CHEMISTRY OF THE NUCLEUS

In a paper dealing with the reproduction of the nucleus it would appear appropriate to devote some space to a consideration of the chemistry of the nucleus. The problems concerned with the submicroscopic structure of the nucleus, the types of protein and nucleic acids detected, and their distribution in and redistribution among the components of the nucleus have received considerable attention during the past few years (for review see Huskins, 12). We know that the chromosome is a fibrous protein, that when it stains with Feulgen's stain it is rich in desoxyribonucleic acid, that the nucleolus is the seat of ribonucleic acid (the two differing in the presence or absence of an oxygen atom), and that heteropycnotic chromosomes or regions retain their desoxyribonucleic acid when the remainder lose their staining capacity.

[27] In certain insects the situation is somewhat complicated by the X being represented by two or more separate chromosomes; the formula for the male is X^1X^2Y, etc.

[28] Certain Lepidoptera appear to have the formula ♂ = XX and ♀ = XY^1Y^2 or XO.

Details of the chemical processes involved in nuclear reproduction are still not understood (Mirsky, 20), but it is evident that this rapidly expanding field will in the future lead to a better understanding of the microscopic units with which we have been concerned.

SUMMARY

A brief account, largely of interest to entomologists only, is given synthesizing the fundamental features of mitosis and meiosis in insects. Chromosome reproduction during prophase is considered to be of universal occurrence. As a general rule the centromere lags behind the body of the chromosome in reproduction by one whole mitotic cycle; at anaphase the chromosomes (apart from their centromeres) are therefore double so that no attraction exists between them. The special case of somatic pairing in the Diptera is treated separately. Here synchronization in the reproduction of the body of the chromosome and its centromere results in single chromosomes after anaphase separation: homologous chromosomes therefore associate in pairs in each and every division. In insects other than the Diptera this synchrony is established only at or by the metaphase of the last gonial mitosis: homologues thus become conjugated, or associated in parallel, in preparation for the intimate pairing, or synapsis of zygotene. The key to the problem of meiotic chromosome pairing is therefore supplied by the Diptera: they constitute the connecting link between ordinary mitosis and meiosis.

REFERENCES

1. Belar, K. Beitrage zur Kausalanalyse der Mitose II. Untersuchungen an den Spermatocyten von *Chorthippus* (*Stenobothrus*) *lineatus* Panz. Arch. f. Entwm., 118 : 359-484. 1929.
2. Belling, J. Chromomeres of liliaceous plants. Univ. Calif. Pub. Bot., 16 : 153-170. 1937.
3. Berrill, N. J., and C. L. Huskins. The "resting" nucleus. Amer. Nat., 70 : 257-261. 1936.
4. Carlson, J. G. Mitotic behaviour of induced chromosomal fragments lacking spindle attachments in the neuroblasts of the grasshopper. Proc. Nat. Acad. Sci., 24 : 500-507. 1938.
5. Darlington, C. D. A cytological demonstration of "genetic" crossing-over. Proc. Roy. Soc. B, 107 : 50-59. 1930.
6. Darlington, C. D. Meiosis. Biol. Revs., 6 : 221-264. 1931.
7. Darlington, C. D. Chromosome mechanics. Bull. Appl. Bot., Gen. and Pl. Breeding, II (no pagination given). 1934.
8. Darlington, C. D. The internal mechanics of the chromosomes, I, II, III. Proc. Roy. Soc. B, 118 : 33-96. 1935.
9. Darlington, C. D. Recent advances in cytology, London, 2nd ed. 1937.
10. Goldschmidt, R., and M. Kodani. The structure of the salivary gland chromosomes and its meaning. Amer. Nat., 76 : 529-551. 1942.
11. Huskins, C. L., and S. G. Smith. Chromosome division and pairing in *Fritillaria meleagris:* The mechanism of meiosis. J. Genet., 28 : 397-406. 1935.
12. Huskins, C. L. Structural differentiation of the nucleus. Reprinted from The structure of Protoplasm, 109-126. 1942.
13. Huskins, C. L. In Press. 1944.
14. Huxley, J. (edited by). The new systematics, London. 1940.
15. Kaufmann, B. P. Somatic mitoses of *Drosophila melanogaster*. J. Morphol., 56 : 125-155. 1934.

16. KLINGSTEDT, H. Digametie beim Weibchen der Trichoptere *Limnophilus decipiens* Kil. Act. Zool. Fenn., 10 : 1-69. 1931.

17. KOLLER, P. C. The internal mechanics of the chromosomes, IV. Pairing and coiling in salivary gland nuclei of Drosophila. Proc. Roy. Soc. B, 118 : 371-397. 1935.

18. MATHER, K. The determination of position of crossing-over, III. The evidence of metaphase chiasmata. J. Genet., 39 : 205-223. 1940.

19. METZ, C. W. Monocentric mitosis with segregation of chromosomes in *Sciara* and its bearing on the mechanism of mitosis, I and II. Biol. Bull., 54 : 333-347. 1933.

20. MIRSKY, A. E. Chromosomes and nucleoproteins. Advances in Enzymology, 3 : 1-34. 1943.

21. POLLISTER, A. W., and P. POLLISTER. The relation between centriole and centromere in atypical spermatogenesis of viviparous snails. Ann. N.Y. Acad. Scis., 45 : 1-48. 1943.

22. SAX, H. J., and K. SAX. Chromosome structure and behaviour in mitosis and meiosis. J. Arn. Arb., 16 : 423-439. 1935.

23. SCOTT, A. C. Haploidy and aberrant spermatogenesis in a Coleopteran, *Micromalthus debilis* Le Conte. J. Morphol., 59 : 485-509. 1936.

24. SHARP, L. W. Introduction to cytology. McGraw-Hill, N.Y. 1934.

25. SHARP, L. W. Fundamentals of cytology. McGraw-Hill, N.Y. 1943.

26. SMITH, S. G. A new form of spruce sawfly identified by means of its cytology and parthenogenesis. Sci. Agric., 21 : 244-305. 1941.

27. SMITH, S. G. Polarization and progression in pairing, II. Premeiotic orientation and the initiation of pairing. Can. J. Research D, 20 : 221-229. 1942.

28. SMITH, S. G., and E. R. BOOTHROYD. Polarization and progression in pairing, I. Interlocking of bivalents in *Trillium erectum* L. Can. J. Research, C, 20 : 358-388. 1942.

29. SMITH, S. G. Techniques for the study of insect chromosomes. Can. Ent., 45 : 21-34. 1943.

30. WHITE, M. J. D. The chromosomes, London. 1937.

31. WHITING, W. P. Multiple alleles in complementary sex determination of *Habrobracon*. Genetics, 28 : 365-382. 1943.

RIBOFLAVIN CONTENT OF MILK PRODUCTS FOR FEEDING PURPOSES[1]

E. V. Evans[2], D. M. Young[3], and H. D. Branion[4]

[Received for publication May 2, 1944]

The use of buttermilk powder, and to a somewhat lesser extent, skim-milk powder, as a component of poultry and swine rations has been extremely widespread for a great many years. More recently, whey powder has also found some use in such rations. These dairy products have served, of course, as valuable protein supplements, supplying part of the animal protein desired in the rations. It was recognized very early that these materials contained other valuable nutritional factors, in addition to the protein. Of these factors, the best known and most intensively studied is riboflavin. Over a considerable period of years, it has been customary to recommend the inclusion of substantial amounts of the dried dairy by-products in poultry rations in order to supply the major portion of the riboflavin[5] requirements.

It is true that the depletion of supplies of milk products available for feeding purposes, together with the tremendously increased demand for poultry and swine production, has resulted, during these war years, in the use of many substitute or replacement materials to maintain the riboflavin levels necessary for high production. In many cases the deficit has been met by the prudent use of other natural feed stuffs, usually of lower potency, but quite frequently it has been necessary to fortify the rations by the use of pure crystalline riboflavin which is available from synthetic production. The milk products, nevertheless, are still very highly regarded and the inadequate supplies are in great demand. Furthermore, it is very probable that there will be some considerable return to the use of these products as they become increasingly available again in the future. Hence it appears that the presentation of the accompanying information which was gathered two or three years ago, should be of interest and value at the present time to both the purchasers of feedstuffs and the processors of dairy products.

The development of assay methods for riboflavin, first of all by biological means but more particularly by the simpler fluorometric and microbiological procedures, has resulted in extensive analyses of the riboflavin content of various types of feeding materials. The literature of the past few years contains numerous reports of such analyses, usually, however, on small numbers of samples of any one type of material, or on

[1] A contribution from the Department of Biochemistry, Ontario Research Foundation, Toronto, and the Department of Animal Nutrition, Ontario Agricultural College, Guelph. The majority of the assays were conducted in the laboratories of the Ontario Research Foundation.

[2] At the time, Research Fellow, Ontario Research Foundation; at present, Assistant Professor of Animal Nutrition, Ontario Agricultural Collage.

[3] At the time, Graduate Student in the Department of Animal Nutrition, O.A.C.; at present, Technical Associate, Connaught Laboratories, University of Toronto.

[4] Head of the Department of Animal Nutrition, O.A.C.; at present, on active service with the Royal Canadian Air Force.

[5] In the earlier years it was the practice to speak of Vitamin G or Vitamin B_2, whereas the chemical name, riboflavin, is now used for the same factor.

special products involved in specific feeding-trials or investigations. Reports by Culton and Bird (2) and by Hodson and Norris (4) are among those which cover somewhat more extensive studies of the riboflavin content of feedstuffs. The former authors reported a range of 17.6 to 29.4 micrograms per gram in 13 samples of dried skimmilk, 12.4 to 29.7 in 5 samples of dried whey, and 31.5 to 39.0 in 10 samples of dried buttermilk. In addition, they found no significant seasonal variation in the riboflavin content of dried skimmilk and buttermilk. Hodson and Norris found a lesser degree of variation in 5 samples of dried skimmilk and 5 samples of dried whey. Most published tables of vitamin values express the content of riboflavin and the other vitamins as "average" values, that is, the average of the large number of values recorded in the literature. Examples of such tables of average vitamin values are those presented by Titus (6) and Ewing (3), and others are to be found in most text books and reviews dealing with animal nutrition. Such values are, at best, valuable only as indications of possible nutritive importance, and, if not used with discretion, may be quite misleading in many cases. If rations are composed on the basis of "average" values, two possible eventualities can be visualized, namely, the wasting of the riboflavin carrier if its potency is in excess of that average figure, or a riboflavin shortage in the ration if the potency of the carrier is below the average. Less frequently encountered are tables which record the observed range of vitamin values for feedstuffs. One example of this type is the table published by Almquist (1), in which the following ranges of values, in micrograms of riboflavin per gram, are recorded: Dried buttermilk 27.3 – 37.8, dried skimmilk 16.1 – 32.4, dried whey 16.2 – 40.0.

Little information has been published on the riboflavin values of milk feedstuffs available in Canada. The work reported here was undertaken in an attempt to remedy this situation and to study the variations in riboflavin potency of products from different plants and from the same plant at different times.

EXPERIMENTAL

In nearly all cases the samples of dried buttermilk, dried skimmilk and dried whey were obtained directly from the producers. In most of the cases in which the samples were obtained from distributors, information on the initial source was furnished with the samples. Most of the samples were from Ontario processing plants, a few were from Quebec plants and only a very few originated elsewhere. Three samples of whey powder and one of skimmilk powder came from United States production which was being marketed in Canada. The majority of sources were represented by single samples, but a number of producers furnished two, three or more samples.

The actual assays of the samples were carried out by a modification of the Snell and Strong (5) microbiological method, in which the extracts were prepared by autoclaving the samples with one-tenth normal hydrochloric acid solution. Subsequent changes in the microbiological procedure have not markedly influenced this laboratory's results on materials of this type, so that the absolute results should compare favourably with those obtained by the more recent procedures. In any event, since the same method was used for all samples covered herein, the relationship of the

values obtained is reliable. Repeat microbiological determinations and assays by a fluorometric method were conducted on a considerable number of the samples and in all cases results agreed closely with the figures from the original microbiological assays, providing further evidence of the reliability of these determinations.

RESULTS

The riboflavin potencies found for the individual samples, together with some information on the samples, are presented in Tables 1, 2, and 3. For purposes of this report, the sources are identified by numbers. These tables require little explanation. It should be pointed out that the results for skimmilk samples 2 and 242 suggest the possibility of an incorrect labelling of these samples at the source, since the values are more typical of the range for buttermilk powder than for skimmilk powder.

TABLE 1.—RESULTS OF ASSAYS OF BUTTERMILK POWDER SAMPLES

Sample no.	Source no.	Date received	When processed	Riboflavin content (Micrograms per gram)
79	3	Feb. 6/41	—	20
121	5	Mar. 8/41	—	33
86	5	Mar. 14/41	Feb./40	32
3	6	Dec. 13/40	—	41
93	6	Jan. 17/41	Dec./40	36
99	6	Mar. 1/41	Jan./40	31
4	7	Dec. 13/40	—	33
72	7	Jan. 17/41	Dec./40 (A)	41
94	7	Jan. 17/41	Dec./40 (B)	27
95	7	Jan. 17/41	Summer/40	22
218	7	May 15/41	Mar./41	34
80	11	Feb. 6/41	—	23
52	16	Jan. 9/41	—	32
50	17	Jan. 9/41	—	48
111	18	Mar. 8/41	—	38
54	20	Jan. 9/41	—	29
82	21	Mar. 1/41	Feb. 15/41	33
81	21	Mar. 1/41	Feb. 20/41	37
57	24	Jan. 9/41	—	39
48	25	Jan. 9/41	—	41
56	26	Jan. 9/41	—	39
96	26	Jan. 17/41	—	27
1	30*	Dec. 5/40	—	41
36	30	Dec. 13/40	—	40
70	30	Jan. 17/41	—	36
71	30	Jan. 17/41	—	33
87	30	Mar. 14/41	—	34
216	30	May 15/41	—	29
238	30	May 15/41	—	34
247	30	June 11/41	—	28
248	30	June 11/41	—	32
259	30	June 11/41	—	30
275	30	July 3/41	—	31
277	30	July 8/41	—	28
278	30	July 8/41	—	23
13	33	Dec. 13/40	—	20
B 6	40	Dec. 13/40	—	45
6	41	Nov. 28/40	—	43
92	41	Jan. 9/41	Dec./40	34
88	41	Mar. 14/41	—	42

TABLE 1.—RESULTS OF ASSAYS OF BUTTERMILK POWDER SAMPLES—*Concluded*

Sample no.	Source no.	Date received	When processed	Riboflavin content (Micrograms per gram)
113	41	April 30/41	Mar. 31/41	32
217	41	May 15/41	April/41	37
258	41	June 11/40	May/41	38
E 5A	41	Sept. 18/41	Aug./41	39
E 9A	41	Oct. 18/41	Sept./41	40
E 18A	41	Nov. 18/41	Oct./41	30
E 29	41	Dec. 18/41	Nov./41	45
E 33	41	Jan. 20/42	Dec./41	45
E 35	41	Feb. 19/42	Jan./42	40
E 40	41	Mar. 23/42	Feb./42	47
49	43	Jan. 9/41	—	26
55	45	Jan. 9/41	—	43
47	49	Jan. 9/41	—	38
E 65	54	June 22/42	—	48
97	55	Feb. 6/41	—	26
B 1	?	June 30/39	—	38
78	?	Feb. 6/41	—	41
276	?	July 3/41	—	31

Range of values: 20 – 48
Distribution: 13 samples 20 – 29
28 samples 30 – 39
17 samples 40 – 48

* The source designated as number 30 was not a producer, and definite information as to the producer was not available. More than one producer may be represented.

The range of riboflavin potencies was quite wide in each of the three types of material. The greatest range was found in the case of buttermilk powder. The distribution of the values for buttermilk powder, shown in Table 1, indicates that the wide range of potencies cannot be attributed merely to one or two samples which deviated from the average. The validity of the range for whey powder is less well established since the number of samples involved was not very large.

In Table 4 is assembled the potency information for all instances in which two or more samples were received from the same producer. It is evident that, in many cases, marked variation in riboflavin potency is encountered in the materials from a single producer.

The limited information available on the processing dates and the type of process employed does not permit of any conclusions on possible variations with season or with processing methods.

Further intensive studies to ascertain and, if possible, overcome, the causes of these wide variations should be of practical importance to both the producers and consumers of milk feeds and such studies are contemplated.

SUMMARY

Fifty-eight samples of buttermilk powder, 35 samples of skimmilk powder, and 9 samples of whey powder were assayed for riboflavin content by the microbiological method. All samples represented feeding materials commercially available in Ontario.

LIBRARY
PURDUE UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
LAFAYETTE, INDIANA

TABLE 2.—RESULTS OF ASSAYS OF SKIMMILK POWDER SAMPLES

Sample no.	Source no.	Date received	Riboflavin content (Micrograms per gram)
101	2	Mar. 8/41	18
75	3	Feb. 6/41	19
66	4	Jan. 17/41	19 (R)*
59	4	Jan. 17/41	17 (R)
63	4	Jan. 17/41	20 (S)
64	4	Jan. 17/41	18 (S)
106	5	Mar. 8/41	16
85	5	Mar. 14/41	16
2	6	Dec. 13/40	40
62	6	Jan. 17/41	19
242	6	May 15/41	38
60	7	Jan. 17/41	19
61	7	Jan. 17/41	20
39	10	Jan. 9/41	19 (R)
76	11	Feb. 6/41	21
45	14	Jan. 9/41	21
40	15	Jan. 9/41	18 (R)
42	17	Jan. 9/41	20 (R)
44	17	Jan. 9/41	21 (S)
125	18	Mar. 8/41	23 (S)
126	18	Mar. 8/41	19 (R)
41	19	Jan. 9/41	17 (S)
43	20	Jan. 9/41	16 (R)
51	25	Jan. 9/41	19 (R)
38	27	Jan. 9/41	19
15	33	Dec. 13/40	21
68	34	Jan. 17/41	21
77	35	Feb. 6/41	18
53	43	Jan. 9/41	20 (R)
33	47	Dec. 13/40	21
37	49	Jan. 9/41	13
74	55	Feb. 6/41	16
279	?	July 8/41	17
280	?	July 8/41	17
281	?	July 8/41	16

Range of values (omitting samples 2 and 242): 13 – 23.

*Letter in bracket designates processing method: R—Roller, S—Spray.

TABLE 3.—RESULTS OF ASSAYS OF WHEY POWDER SAMPLES

Sample no.	Source no.	Date received	Riboflavin content (Micrograms per gram)
46	39	Jan. 9/41	14
65	39	Jan. 17/41	16
67	39	Jan. 17/41	16
E34	52	Jan. 26/42	27
E54	52	Mar. 5/42	20
E55	52	Mar. 5/42	22
E45	56	May 11/42	24
E46	56	May 11/42	22
E50	57	May 17/42	20

Range of values: 14 – 27

TABLE 4.—VARIATIONS IN DRIED MILK PRODUCTS FROM SAME PRODUCER

Product	Producer	Number of samples	Range of riboflavin values (Micrograms per gram)
Buttermilk powder	6	3	31 – 41
	7	5	22 – 41
	21	2	33 – 37
	41	13	30 – 47
Skimmilk powder	4	4	17 – 21
	5	2	16
	6	3	19 – 40*
	7	2	19 – 20
	17	2	20 – 21
	18	2	19 – 23
Whey Powder	39	3	14 – 16
	52	3	20 – 27
	56	2	22 – 24

* See note in text regarding samples 2 and 242.

Wide variations in the riboflavin content of similar products were found. In many cases, similar products from a single producer showed marked variations in riboflavin potency.

ACKNOWLEDGMENT

The authors wish to express their thanks to the many producers and distributors for their kind co-operation in making available the samples of dried milk products involved in this survey.

REFERENCES

1. ALMQUIST, H. J. Meeting the riboflavin requirements of chickens. Feedstuffs, Feb. 20, 1943.
2. CULTON, T. G. and H. R. BIRD. The riboflavin content of poultry feedstuffs. Poultry Science 20 : 3. 1941.
3. EWING, W. R. Handbook of Poultry Nutrition, 2nd edition. 1943.
4. HODSON, A. Z. and L. C. NORRIS. A fluorometric method for determining the riboflavin content of feedstuffs. J. Biol. Chem. 131 : 621. 1939.
5. SNELL, E. E. and F. M. STRONG. A microbiological assay for riboflavin. Ind. Eng. Chem. Anal. Ed. 11 : 346. 1939.
6. TITUS, H. W. Practical feeding of poultry. U.S. Dept. of Agr. Year Book, 1939, pages 819-843.

THE YIELD AND OTHER CHARACTERS OF INBRED LINES AND SINGLE CROSSES OF SUNFLOWERS

J. Unrau[2] and W. J. White[3]

Dominion Forage Crops Laboratory, Saskatoon, Sask.

[Received for publication May 2, 1944]

The original selections of sunflowers secured by the Dominion Forage Crops Laboratory consisted of a great variety of types, and their progenies were very variable. In the attempt to secure homzygosity for such characters as high oil content, non-branching, low hull percentage, etc., inbreeding was practised. It was soon evident that while the objective of increased uniformity was being attained, vigour was being sacrificed. Hybrids between inbred lines produced by hand pollination appeared so promising that as the next step a procedure similar to the hybrid corn technique was utilized for producing single crosses on a fairly extensive scale. It is the purpose of this paper to report the results of inbreeding and of preliminary studies with hybrids.

LITERATURE REVIEW

The utilization of the hybrid corn technique for increasing sunflower seed yields was suggested in Russia as early as 1934 by Morozov (4). He used isolation plots for natural crossing of inbred lines and obtained crossing of from 15.6 to 77.8%. He reported increases in seed yield of up to 22% over the original parental varieties.

Jagodkin (2), another Russian worker, reported obtaining lines pure breeding for disease and insect resistance, strong single stems, high oil content, and other characters as a result of selective inbreeding. By hybridization of these resistant lines he obtained seed yields above those of standard varieties. He also suggested the utilization of the hybrid corn technique to increase commercial yields of this crop.

The effect of inbreeding and crossing sunflowers is similar to that experienced with other naturally cross pollinated crops. Kirk (3) and later Tysdal *et al.* (7) showed that marked reductions in forage and seed yields followed the inbreeding of alfalfa. The latter reported significant increases of both forage and seed yields in F_1 hybrids compared to standard varieties. Hayes and Schmid (1) reported that the F_1 of crosses between inbred lines of brome-grass yielded 126.5 to 220.9% of the commercial check. Although having a direct bearing on the problems of producing hybrid sunflowers, the literature dealing with hybrid corn is too extensive to review here.

[1] Contribution from the Division of Forage Plants, Experimental Farms Service, Dominion Department of Agriculture, Ottawa, Canada.

[2] Agricultural Assistant.

[3] Officer-in-charge.

MATERIALS AND METHODS

Inbreeding Study

Of 207 lines obtained from open fertilized single head selections made from unimproved Mennonite material in 1937, because of the presence of undesirable characters or the lack of sufficient self-fertility, all but 4 lines had been discarded by 1942. In the case of only 2 of these 4 lines was there sufficient reserve seed of the original selection and each of 4 subsequent inbred generations to enable the laying down of a triple rod row plot with 3 replicates. A split plot design was used in which the 2 strains constituted the main plots and the different generations the sub-plots. Reserve selfed seed of plants selfed in each generation was bulked to represent that generation of the line. Rows were spaced 3 feet apart with plants in the rows being from 5 to 8 inches apart, this spacing being recommended for commercial production (6). Seeding was done with the Columbia seeder to a depth of about 2 inches. Unless otherwise stated, the seeding and thinning after emergence was done as is described by Putt and Unrau (6).

Crossing Study

In 1940, two plants in 1 line inbred for 3 generations were reciprocally crossed with 2 plants from another line having like inbreeding history. Crossing was done without emasculation, pollination being performed periodically until flowering on each head was complete. Enough crossed seed was obtained from each of the 2 lines for a single rod row, 6 replicate test in 1941. In 1941 these same lines were hand crossed but in addition an isolated plot was set out in which the 2 lines were sown in alternate rows and allowed to cross naturally. The seed from the natural crossing plot was used to establish a test at both Saskatoon and the Dominion Experimental Station at Swift Current in 1942.

In 1942 isolated plots were set out involving in pairs various combinations of Sunrise and 3 inbred lines of Mennonite. Some of these were unsuccessful since flowering did not occur simultaneously. A successful plot was, however, obtained for the 2 lines used in the 2 previous years. In 1943 tests were conducted involving this and other crosses. In all tests data were obtained on the yield of seed calculated in pounds per acre. Measurements on plant height and head diameters were taken at maturity on 5 random plants in each plot, the average of these separate measurements being used as the plot value. Seed size as indicated by 1000 kernel weight was measured by weighing three lots of 100 seeds from each plot. Weight per bushel was obtained when sufficient seed was available. Percentage kernel to hull was obtained in the years 1941 and 1942 by hand separation on a 5-gram sample from every plot. In 1943 this determination was made with a mechanical huller using a 10-gram sample per plot.

Oil analysis, based on the dry weight of the whole seed, was obtained on a composite sample made up from seed from each replicate of a treatment. The determinations were made by the Oil Seeds Laboratory, University of Saskatchewan, Saskatoon.

RESULTS AND DISCUSSION

Inbreeding Study

A summary of the data from the inbreeding study conducted in 1942 is presented in Table 1.

TABLE 1.—INFLUENCE OF FOUR CONSECUTIVE GENERATIONS OF INBREEDING ON THE YIELD OF SEED AND OTHER CHARACTERS IN TWO LINES OF SUNFLOWERS

(A = S-37-$\overline{25}$, B = S-37-$\overline{49}$)

Generation of inbreeding	Yield per acre			Plant height			Head diameter			100 seed wt.	
	Line A	Line B	Av. 2 lines	Line A	Line B	Av. 2 lines	Line A	Line B	Av. 2 lines	Line A	Line B
	lb.	lb.	lb.	in.	in.	in.	in.	in.	in.	gm.	gm.
Orig. open	2187	2030	2108	53.7	55.0	54.4	8.3	8.4	8.4	77	73
1st	1777	940	1358	50.0	47.7	48.8	8.0	6.8	7.4	78	65
2nd	1037	1190	1114	45.3	44.0	44.6	6.6	6.9	6.8	87	64
3rd	1057	993	1025	46.3	40.3	43.3	6.3	7.4	6.8	71	56
4th	797	877	837	44.3	47.7	46.0	6.0	6.8	6.4	71	52
Line Av.	1371	1206		47.9	46.9		7.0	7.2		76.8	62.0
L.S.D.* between generations between 5% point			365			5.4			.47		
generations 1% point			502			7.4			.65		

* Least Significant Difference.

The most significant effect of inbreeding on vigour is indicated in the data for seed yield. As an average of both lines a reduction in yield amounting to 35.6% and exceeding odds of 99 : 1 for significance resulted from 1 generation of inbreeding. Four generations of selfing decreased the yield by 60.3% as an average of both lines. The reduction in yield was much less in the later generations of inbreeding, there actually being a significant difference only between the fourth and the first generation. This levelling off of the loss in yield in later generations is to be expected if it is assumed that yield and vigour are proportional to the heterogeneity of the material.

Analyses of variance showed a significant interaction of lines × generations. It is obvious from the Table 1 that while the decline in yield of the 2 lines is similar from the second generations onward, Line B showed a much greater drop than Line A as the result of the first generation of inbreeding.

Significant effects of inbreeding are noted also in the data for plant height and head diameter. These characteristics give an indication of plant vigour, and it is evident that for both characters there is a reduction exceeding the 1% point for significance as the result of 4 generations of inbreeding. Again the greatest reduction occurs in the early generations and there is also a levelling off tendency in the later generations. As in the case of yield, Line B had a greater drop in the first generation in height and head size than Line B.

Since small seed size is considered desirable, the effect of selection on seed size is of interest. Line A is large seeded and highly self-sterile. It appeared that only the large seeded plants gave sufficient seed to carry on the line. Therefore seed size is fairly similar in all generations. Line B, on the other hand, was fairly self-fertile and selection for small seed size

was possible, with the result that there was a more or less progressive decrease in size with each advance in generation of inbreeding.

The later inbred generations within a line were highly uniform in plant type, stem strength, head shape, seed colour and other visible characteristics. At the same time between lines there were some fairly obvious differences of visible characters. An indication of the variability in the original open pollinated selection as contrasted to the relative uniformity of the fourth generation inbred progeny of that selection may be seen in Figure 1.



FIGURE 1. Comparison of open fertilized selection (on left) and 4th generation inbred of the same line (on right). Note variability in plant height and stem strength of open fertilized row as contrasted to the uniformity for those characters in the inbred, which was also uniform for head shape, pigmentation of leaves, flowers and seeds.

Crossing Studies

The F_1's of 2 inbred lines crossed reciprocally by hand without emasculation in 1940, were tested in comparison with the inbred parents in 1941. A summary of the data from this test is presented in Table 2.

The most significant feature of the data in Table 2 is the high seed yield of the single crosses. As an average the reciprocal crosses yielded 247% of the average of the inbred parents. Unfortunately no Mennonite material was included in this test as a check, but the average yield of Mennonite grown under similar conditions in an adjacent 6 replicate test was 830 pounds per acre. The single crosses, therefore, yielded 60.8% more than the Mennonite variety in this adjacent test.

With respect to other characters, the effect of hybrid vigour is clearly seen in the significantly greater height, head diameter, and seed size of the single crosses compared to the parent inbreds. Percentage kernel was

somewhat lower in the F_1's. It should be pointed out that while the values for seed size were higher for the hybrids than for the inbred lines, this was largely due to the poor seed development of the inbreds and the more normal seed development of the single crosses. With regard to percentage oil the hybrids, while a little lower than their parents, were higher in oil than any Mennonite or Sunrise material in that year, indicating that obtaining a combination of high seed yield coupled with high oil percentage is possible. In this test there were no actual counts made to determine the proportion of selfs to hybrids, but very few selfs were observed in the hybrid rows.

TABLE 2.—COMPARISON OF YIELD AND OTHER CHARACTERS OF TWO PARENT INBRED LINES AND THEIR RECIPROCAL SINGLE CROSSES PRODUCED BY HAND POLLINATION

Material	Yield per acre	Height	Head diam.	1000 seed wt.	Percentage kernel	Percentage oil
	lb.	in.	in.	gm.	%	%
Inbred Line B*	543	31.2	3.1	27.4	73.3	36.9
Inbred line C†	539	39.8	3.9	24.7	65.6	36.4
F_1 B × C	1320	44.5	4.9	47.9	64.9	35.6
F_1 C × B	1349	41.4	5.3	46.7	64.5	34.5
Nec. diff. odds 19 ÷ 1	564	3.0	0.9	15.8	5.8	

* = Line S-37-49. † = S-37-388.

In 1941, Lines B and C were put in an isolated natural crossing plot. The determination of the degree of natural crossing was possible because Line B carries a factor, or factors, for purple seed coat colour the expression of which is inhibited when selfed but which on crossing is expressed irrespective of the direction of the cross. In 1942 the percentage of hybrids was found to be 57.6 in the single cross of B X C and 80.8 in the single cross of C × B. Both inbred lines are about equally self fertile, but Line B is a heavier pollen producer than Line C which fact may account for the difference noted above.

Data on the yield and other characters of single crosses in tests at Saskatoon and Swift Current are presented in Table 3.

As in the previous year's test the single crosses were very significantly superior in yield as compared to the inbred parents. In addition they greatly outyielded the Mennonite and Sunrise checks. The comparatively high yield of inbred Line B is somewhat puzzling, but it may be that the season was especially favourable for the development of this line. On comparing the 2 single crosses it appears that yield bears some relationship to the extent of natural crossing. The F_1 of C × B with 80.8% crossing significantly outyielded the F_1 of B × C in which only 57.6% crossing occurred. At the same time it should be pointed out that the single cross of B × C outyielded by odds in excess of 99 : 1 both of the inbreds and the 2 varieties, the actual increase over Mennonite being 47.4%. With respect to the other characters heterosis is again evident in the greater

TABLE 3.—AVERAGES FOR YIELD AND OTHER CHARACTERS OF SINGLE CROSSES PRODUCED BY NATURAL CROSSING AND TESTED AT SASKATOON AND SWIFT CURRENT, 1942

Material	Yield per acre	Plant height	Head diameter	1000 seed wt.	Weight per bushel	Percentage kernel	Percentage oil
	lb.	in.	in.	gm.	lb.	%	%
Mennonite	998	55.5	4.8	60.1	26.6	53.4	29.2
Sunrise	361	50.8	4.8	27.9	22.2	53.9	25.4*
Inbred B	1177	48.6	5.2	46.0	25.5	60.0	31.0
Inbred C	351	43.2	4.2	30.0	21.5	63.0	32.2
F_1 B × C	1471	56.4	5.8	60.2	27.4	56.6	30.6
F_1 C × B	1606	59.4	5.5	60.9	27.5	54.9	30.2
Nec. diff.							
5% point	134	2.2	0.6	4.3	1.2	1.4	
1% point	182	2.9	0.8	5.8	1.7	1.8	

* Unduly low for Sunrise because of killing frost before maturity.

height, larger head size, and larger seed of the single crosses. They also display a very desirable oil content and satisfactory kernel percentage.

As pointed out previously several single crosses were made in 1942 by natural crossing technique. These were included in tests in 1943 at several experimental stations on the prairies. The data from the test at Saskatoon are given in Table 4.

TABLE 4.—COMPARISON OF 5 SINGLE CROSSES WITH MENNONITE, SUNRISE AND 1 INBRED PARENT TESTED AT SASKATOON, 1943

Material	Yield per acre	Plant height	Head diameter	1000 seed wt.	Weight per bushel	Percentage kernel	Percentage hybrids
	lb.	in.	in.	gm.	lb.	%	%
Sunrise	223	40.7	4.1	28.4	29.6	56.0	
Mennonite	976	51.3	4.1	52.4	29.3	58.0	
Inbred B	566	40.3	5.1	35.3	31.4	59.0	
F_1 C × B	1285	48.0	4.6	44.8	34.2	61.0	89.0
F_1 C × Sunrise	1405	45.3	6.0	47.4	32.9	62.0	95.2
F_1 Sunrise × C	691	44.0	4.7	42.0	31.0	59.0	33.2
F_1 Sunrise × B	811	43.3	5.5	45.0	31.0	58.0	43.6
F_1 D × C	609	40.7	4.2	38.1	31.3	62.0	
Nec. diff. 5% point	518	N. sig.	N. sig.	7.19	*	3.9	

* Insufficient seed to analyse on single plot basis and therefore no variance analysis is possible.

While Table 4 shows that the single crosses differed greatly in yielding ability it is evident that those which showed a high percentage crossing (C × B and C × Sunrise) were substantially higher in yield than all other material in the test. The data again indicates a relationship between yield and percentage crossing. The yield per acre of C × Sunrise with 95.2% crossing was 1405 pounds while that of Sunrise × C with 33.2% crossing was only 691 pounds per acre. Similarly the cross Sunrise × B

with 43.6% crossing was not outstanding. However, the relationship between percentage crossing and yield was exaggerated by the low yield of Sunrise. This variety yielded poorly because of frost damage before maturity, therefore the Sunrise selfs and sibs occurring in the single crosses contributed in only a small degree to the yield. This is shown by the fact that seed harvested from the cross Sunrise × B showed that 96.8% was from hybrid plants. In addition to the frost damage, competition from the more vigorous and earlier maturing hybrid plants depressed the yield of Sunrise selfs and sibs.

The low percentage crossing obtained in some of the single crosses involving Sunrise is partially accounted for by the lack of simultaneous flowering of the members of paired lines in the natural crossing plots. In addition, it has been observed that Sunrise is a heavy pollen producer and more self-fertile and consequently makes a poorer female parent than other lines.

No concrete explanation can be offered for the comparatively poor performance of the single cross D × C. It was impossible to determine the percentage crossing in this material but since Line C, as pointed out above, is a poor pollen producer the crossing percentage may have been low. It is also possible that Line D has poor combining ability. With the information available it is impossible to determine whether either or both of these factors provide an adequate explanation of the poor yielding behaviour of this cross.

In previous years plants of the single cross C × B had been taller growing and had larger heads than Mennonite or the inbred lines. In 1943 there was very little relationship between height, head diameter and yield. It should be pointed out that for ease of harvesting, relatively short growth is desirable and in this respect the single crosses are just as satisfactory as Mennonite. Contrasted to Mennonite the high degree of uniformity of the single crosses for height, head size, stem strength, and seed size is definitely desirable.

In 1943, tests involving single crosses, Sunrise, Mennonite originating from different locations, and three 1942 Scott selections were conducted at a number of experimental stations in the Prairie Provinces. A summary of the yield data is presented in Table 5.

Variance analysis showed highly significant differences between the material in the tests. Varieties × stations interaction was also highly significant, indicating that the different varieties and single crosses reacted differently at the different stations. The performance of the single crosses in some of these tests is somewhat disappointing. However, it should be kept in mind that in only the cross C × B was there a relatively high percentage crossing (89.0%). As an average of all tests this single cross gave the highest yield and significantly outyielded all but 2 strains. Although at 3 stations the yield of C × B was lower than some other strains the differences were not significant. As pointed out in the case of the Saskatoon test the yields of single crosses Sunrise × C, and Sunrise × B were unduly depressed by the presence of high proportions of Sunrise selfs and sibs. The low yield of the cross Sunrise × E is probably largely due to the fact that very few hybrid plants were observed in this single cross, and practically

TABLE 5.—SEED YIELDS FROM THE STATION TESTS OF VARIETIES AND SINGLE CROSSES.

Material	Yield (lb. per acre)							Grand average
	Sask-atoon	Scott	Swift Current	Mel-fort	Indian Head	Leth-bridge	Mor-den	
F_1Sunrise × E*	392	28	336	227	694	387	1986	579
F_1D × C	609	378	1290	1451	908	941	2107	1098
F_1Sunrise × C	691	189	950	1121	719	911	2249	976
F_1C × B	1285	521	1304	1834	920	1204	2493	1368
F_1 Sunrise × B	811	260	1408	935	850	695	2402	1052
Menn. (Rost. orig.)	1182	433	1295	1878	886	1047	2072	1256
Menn. (Man. orig.)	952	409	1191	1723	883	671	2209	1148
Menn. (Winkler, Man.)	691	161	967	1337	418	1358	2219	1024
Sunrise	174	74	251	204	856	418	2054	576
Scott Seln. No. 1	926	493	1182	1917	1121	914	1997	1221
Scott Seln. No. 2	1463	363	1059	1735	958	817	1917	1185
Scott Seln. No. 3	1175	410	1119	1737	417	794	2353	1143
Nec. diff. 19 : 1 odds	325	194	319	351	N. sig.	336	N. sig.	169
Nec. diff. 99 : 1 odds	444	263	436	480		460		219

* = S-488 a line very similar to Sunrise introduced from Russia.

all the harvested seed was from Sunrise plants. Reliable information on the yielding ability of these various combinations of inbred lines in single crosses can be obtained only when combinations having a high percentage crossing are secured.

CONCLUSIONS

Although the data presented show that inbreeding brings about a lowering of yield and vigour in sunflowers, inbreeding is of value in eliminating undesirable genes and in bringing about uniformity for desirable characters such as non-branching strong stems, earliness, small seed size, and high oil content. Because of their low yield inbred lines will probably not be of value as commercial varieties. Morozov and Ananeva (5) of the Saratov Experimental Station report that during 19 years of inbreeding sunflowers no strains materially superior to the original forms had been obtained by selective inbreeding alone. As in the case of corn, the value of inbreeding sunflowers appears to be for production of lines breeding true for desirable characters which can then be used in combinations to produce single cross hybrids.

The high yield of single crosses is contingent upon securing a high degree of crossing between the paired inbred lines. To accomplish this the selection of lines with similar flowering periods or the controlling of time of flowering by staggering seeding dates is essential. Unless the paired lines differ in pollen yield and, or, self-fertility it appears that both inbred lines will cross to about the same extent and therefore seed from both could be used commercially. If one line of a pair is a heavy pollen producer and fairly self-fertile it may not cross sufficiently to warrant using its seed with the reciprocal for the production of the commercial crop.

As far as possible it would be desirable to have one or both lines carry marker genes so that the extent of crossing could be determined. Unless this determination is possible in the seedling stage it would be advisable

to withhold distribution of the hybrid seed for commercial production for a year to permit the determination of the degree of crossing. If the marker characters are displayed in the seedling the determination of extent of crossing could be done in greenhouses in the winter months.

It may be possible to offset the effect of fairly low percentage crossing by increasing the seeding rate. This has been indicated in the data presented but a good deal of further work needs to be done on this problem.

A word should be said about the feasibility of the use of double crosses. This would be very desirable since larger yields of crossed seed would be obtained. While as yet no attempt has been made to produce double crosses, it has been noticed that F_1 hybrids are very self and possibly sib-fertile. This would likely limit the extent of natural crossing between two isolated single crosses and result in a fairly low proportion of double crossed seed. However, it is impossible to reach any definite conclusions on this question until it has been investigated.

SUMMARY

1. The open fertilized progeny of 2 selections were compared to the 1st to 4th generation inbred progenies of these selections using a randomized split plot design with 3 replicates. Four generations of inbreeding reduced the average yield of the 2 selections by 60.3%. The greatest drop in yield occurred in the first generation being 35.6%. Inbreeding in a similar way reduced plant height and head size.

2. Selective inbreeding resulted in a high degree of uniformity for such desirable characters as date of maturity, stem type and strength, seed size, percentage oil, and other characters.

3. Reciprocal single crosses made by hand pollination between 2 inbred lines outyielded the average of the inbred parents by 247%. The increase over Mennonite grown in an adjacent test was 60.8%. The same single cross obtained by natural crossing outyielded Mennonite by 47.4%.

4. Crossing as high as 95.2% has been obtained in isolated natural crossing plots. Differences in date of flowering of the 2 lines being crossed was apparently one of the main causes for a low extent of natural crossing. By later seeding of the earlier flowering line it is possible to obtain simultaneous flowering.

5. Differences in pollen productivity and the degree of self fertility of the 2 lines also appear to influence the extent of natural crossing.

6. An indication of a positive relationship between extent of natural crossing and yield was found. In commercial production it would be desirable to determine the degree of crossing obtained before releasing the seed to growers.

ACKNOWLEDGMENTS

The earlier inbreeding and selection and the hand pollinated single crosses were made under the direction of Mr. E. D. Putt. His contribution to this study is gratefully acknowledged.

REFERENCES

1. Hayes, H. K. and A. R. Schmid. Selection in self pollinated lines of *Bromus inermis* Leyss., *Festuca elatior* L., and *Dactylis glomerata* L. Jour. Amer. Soc. Agron. 35 : 934-943. 1943.

2. Jagodkin, I. G. Application of method of inbreeding and diallel crossing in sunflower growing. Plt. Brdg. Abst. Vol. 8, Page 65, 1937-38. (Original not seen).

3. Kirk, L. E. Self-fertilization in Relation to Forage Crop Improvement. Sci. Agric. 8 : 1-35. 1927.

4. Morozov, V. K. Practical utilization of phenomenon of heterosis in sunflowers. Plt. Brdg. Abst. Vol. 5, Page 143, 1934–35. (Original not seen)

5. Morozov, V. K. and S. V. Ananeva. Results of the application of inbreeding to sunflowers at the Saratov Breeding Station. Plt. Brdg. Abst. Vol. 9, Page 106, 1939. (Original not seen.)

6. Putt, E. D. and Unrau, J. The effect of various cultural practices on seed and plant characters in the sunflower. Sci. Agric. 23 : 384-398. 1943.

7. Tysdal, H. M.; Kiesselbach, T. A. and H. L. Westover. Alfalfa Breeding. Coll. of Agric. Univ. of Neb. Res. Bull. 124. 1942.

TREATING, PACKAGING AND STORING POTATO EYE SETS[1]

J. W. MARRITT[2]

Dominion Plant Inspection Office, Edmonton, Alberta

[Received for publication January 20, 1944]

Eye sets are becoming an increasingly important factor in the seed potato trade of the Prairie Provinces, mainly because they can be widely distributed at little cost. Seed houses can ship eye sets by express, parcel post, or airmail, and sets from certified seed potatoes are made available in this way to many potato growers who do not have ready access to the regular certified seed potato trade.

The biggest demand for eye sets has been from farmers who grow potatoes only for their own use. A farmer can buy a 50-eye set package for a nominal outlay and produce from 50 to 100 pounds of good seed potatoes for planting the following year.

The eye set has a large cut surface which exposes it to deterioration from fungi, bacteria, and excessive loss of moisture. The methods that have been used by seed houses in preparing, treating and packaging eye sets to prevent deterioration have not always been successful, and the sets have not always given satisfactory results. Scannell (2) at Indian Head found that yields from eye sets of the same variety but from different sources varied widely, and subsequent tests at Edmonton gave similar results. To assist seed houses in developing an assured method of handling eye sets, experiments were started at Edmonton in 1937 by the District Inspector Seed Potato Certification, Dominion Department of Agriculture and as the work progressed from year to year the following phases of the problem were studied:—

1. Treating potatoes before eye sets are cut.
2. Size of eye set.
3. The best treatment of eye sets after they are cut and before packaging.
4. The most suitable package for eye sets.
5. The effect of storage temperature on the keeping quality of eye sets.

MATERIALS AND METHODS

Early Ohio and Carter's Early Favorite potatoes from fields that passed inspections for seed certification were used throughout the experiments.

A vegetable baller was used to cut $\frac{1}{2}$–ounce eye sets approximately $\frac{7}{8}$ inch in depth.

A wax-coated carton, wrapped with waxed kraft paper was used as the standard package. This held 25 eyes, which represented one replicate

[1] Contribution No. 40, Plant Protection Division, Science Service, Dominion Department of Agriculture.

[2] District Inspector, Plant Diseases, Plant Protection Division, Dominion Department of Agriculture, Edmonton, Alta.

in the test. The eyes were planted 18 inches apart, in rows 3 feet apart. A simple randomized plan was used for each experiment. Ten replicates were made for all tests except in 1941 and 1942, when only 5 replicates were planted.

RESULTS

Treating the Tubers before Eyes Are Cut

Tests were made on the treatment of tubers with an organic mercury dip in 1940, 1941 and 1942. The eye sets were then cut and stored from 3 to 4 weeks at 60° F. to 65° F. before being planted.

The tubers used in the 1940–41 tests were taken from a crop in which storage rot was present before grading. No storage rot was present in the crop from which the 1942 tubers were selected.

TABLE 1.—TUBER TREATMENT

Treatments	1940		1941		1942	
	Stand	Average yield of 10 replicates	Stand	Average yield of 5 replicates	Stand	Average yield of 5 replicates
	%	lb.	%	lb.	%	lb.
Tubers treated and eye sets washed	—	—	96.0	39.2	98.4	69.6
Tubers treated and eye sets dusted	44.0	30.8	—	—	98.4	70.6
Tubers not treated and eye sets washed (check)	—	—	48.8	24.6	97.2	67.8
Tubers not treated and eye sets dusted (check)	0.0	0.0	16.8	6.2	96.8	66.0

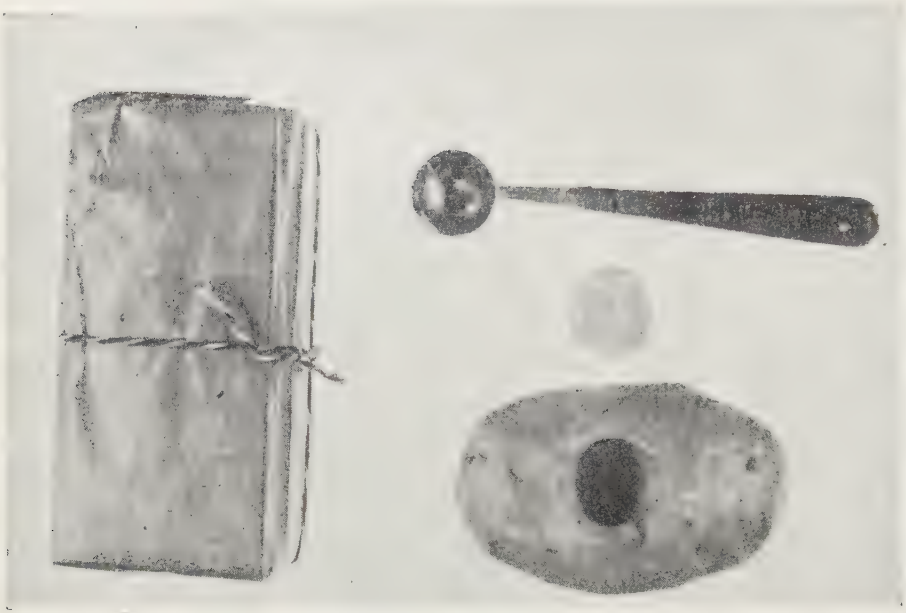
Potato eyes cut from tubers that were treated with an organic mercury dip in 1940–41 gave significantly better stands and yields than eyes cut from untreated tubers. Optimum growing conditions, and the absence of storage rot in the potato stock in 1942 gave satisfactory stands and yields from eye sets from both treated and untreated tubers; but even under those optimum conditions, potato eyes cut from treated tubers gave the better results.

Size of Eye Sets

As eye sets are shipped by parcel post, and sometimes by air mail they must not be too large for economic distribution, but they should be cut large enough to ensure a satisfactory crop. If the eye set is too small, there is not only a greater danger of deterioration from loss of moisture, but yields are definitely decreased. Bushnell (1) summarizes a review of literature on potato yields as affected by size of sets, as follows:

"There is in general a gradual decrease in yield from the largest seed piece tested to seed pieces $\frac{1}{2}$ ounce in size. There is a much greater decrease in yield as you decrease the weight of a set below this point."

This indicates that if the best results are to be obtained, it is important to cut eye sets about a $\frac{1}{2}$ ounce in weight. Table 2 gives the results of the test made in 1937 with different weights of eye sets.



A wax coated carton wrapped with wax kraft paper as shown above, is a satisfactory package for storing and shipping eye sets. The tool used to cut the eye sets is shown on the right.



The eye sets on the right are dusted with ground limestone while those on the left are washed under running water.

TABLE 2.—DIFFERENT WEIGHTS OF EYE SETS

Weight in grams and percentage of 1 ounce	Stand	Average yield of 10 replicates
	%	lb.
15 grams—53%	92.8	50.5
12 grams—42%	91.6	50.8
9 grams—32%	81.6	42.9
6 grams—21%	88.4	41.5
3 grams—11%	81.6	37.0
2 × S.E. of Diff.	1.8	4.1

The test indicates that the depth, as well as the weight of the eye set is important. The 9-gram and the 3-gram sets were shallow and gave the lowest yields in relation to weight. Further tests in 1938 indicated that it was necessary to cut the eye sets at least $\frac{7}{8}$ inches in depth to obtain good results.

Treatment of Eye Sets

Slaked lime, ground limestone, and magnesium limestone have been used by seed houses to dust potato eyes after they were cut and before packaging, and these materials were tested, as well as other dusts. Slaked lime proved unsatisfactory, as it sometimes injured the sets, while flours of sulphur decreased the vigour of the sets which resulted in lower yields. Ground limestone and magnesium limestone were among the most satisfactory materials tested.

TABLE 3.—TREATING EYE SETS

Treatment	Stand	Average yield of 5 replicates
	%	lb.
Washed in water	76.8	31.2
Traumatic acid 1-1000	76.8	33.8
Traumatic acid 1-2000	72.0	27.2
Washed in water and dusted	55.2	23.4
Traumatic acid 1-1000 dusted	52.0	21.3
Traumatic acid 1-2000 dusted	48.8	19.6
No treatment	49.6	19.5
Dusted	40.8	20.8

In 1940 traumatic acid, a hormone that stimulates cork formation was tested, but without definite result. The test, however, indicated the increased value of washing potatoes in water over other treatments.

Experiments were designed in 1941 and 1942 to determine more fully the value of washing potato eyes in water before packaging. A check treatment of chloride of lime and distilled water was included, as Edmonton tap water, which was used, contains a small amount of chloride of lime.

TABLE 4.—WASHING EYE SETS

Treatment	1941		1942	
	Stand	Average yield of 20 repl.	Stand	Average yield of 5 repl.
	%	lb.	%	lb.
Chloride of lime	88	33.6	98.4	68.4
Washed in water	88.0	32.2	97.2	67.8
Washed under running water	87.0	31.4	—	—
Washed in distilled water	84.2	32.8	95.2	62.2
Untreated	61.0	17.9	92.0	62.0
Dusted with magnesium limestone	—	—	96.8	66.0

There was very little difference in the lots that were washed in the 1941 tests. All check plots planted with eye sets that were untreated, however, gave significantly lower yields.

In the 1942 test, all treatments, including the dusted and untreated checks, produced good stands and yields. This was probably due to the optimum growing conditions that prevailed, and the absence of storage rot in the stock from which the eye sets were cut, and indicates that under favourable conditions dusting and packaging the eye sets as cut will give as good results as washing. The tests in 1940 and 1941, however, indicate that washing the potato eyes gives better results under less favourable conditions. It is, therefore, a more reliable treatment.

Most Suitable Package for Potato Eyes

Moisture-tight packages have proved to be the most suitable containers for eye sets. Sets packed in such containers do not lose weight from loss of moisture, and the vitality of the set is maintained. The danger of rapid deterioration due to fungi and bacteria is also less.

In 1939 and 1942 the differences in stand and yield were not significant, because conditions were favourable. In 1939 the packages of eye sets were held under ideal storage temperatures. In 1942 lack of storage rot

TABLE 5.—PACKAGE TEST

Type of package	1938		1939		1940		1941		1942	
	Stand	Average yield	Stand	Average yield	Stand	Average yield	Stand	Average yield	Stand	Average yield
	%	lb.	%	lb.	%	lb.	%	lb.	%	lb.
Wax coated carton with wax kraft paper wpd.	96.8	77.0	84.0	16.0	98.0	46.9	82.0	39.0	99.2	68.4
Wax coated carton only	—	—	79.6	15.6	—	—	42.4	28.0	—	—
Carton not wax coated or wax kraft wrapped	52.8	37.0	75.6	14.1	20.4	16.7	25.6	15.0	94.4	64.0
2 × S.E. of Diff.		11.2				6.2		3.7		

in the stock that was used also had a favourable influence on the keeping quality in all types of containers. In 1940 and 1941 storage rot was present in the stock used, and significant differences were obtained.

Storage Temperature for Potato Eyes

In the 1938 experiments it was found that packages of potato eyes stored under different temperatures showed definite differences in keeping quality, especially when the storage period extended to two or three weeks. In 1939 a test was made with storage temperatures of 77° F., 68° F., 59° F. and 50° F. This test has been continued each year since, with a 38° F. test included in 1941 and 1942.

TABLE 6.—STORAGE TEMPERATURES

Storage temperature	1939		1940		1941		1942	
	Stand	Average yield	Stand	Average yield	Stand	Average yield	Stand	Average yield
	%	lb.	%	lb.	%	lb.	%	lb.
77° F.	95.6	17.6	88.0	45.5	94.4	30.7	96.0	59.0
68° F.	75.6	13.4	3.2	2.0	80.8	31.1	97.6	62.4
59° F.	61.2	10.6	6.8	4.0	48.6	21.1	98.4	57.4
50° F.	70.4	12.6	5.2	4.0	71.2	22.2	99.2	63.4
38° F.	—	—	—	—	85.6	26.4	98.4	63.4
2 × S.E. of Diff.		3.78				3.86		

This test indicated that 77° F. was the most reliable, and 59° F. the most unreliable storage temperature. The favourable results obtained with the 77° F. storage were probably due to the more rapid cork formation.

CONCLUSION

The following procedure is recommended as a result of the experiments that have been conducted:

(1) Carefully select the certified seed stock from which the eyes are to be taken. Only stock that has been free during storage period from frost and storage rot should be used. The best time to find this out is before the potatoes have been graded.

(2) Treat tubers before eye sets are cut. Good results have been obtained from an organic mercury dip.

(3) Use a cutting tool that will cut an eye set at least $\frac{7}{8}$ inch in depth and $\frac{1}{2}$ ounce in weight.

(4) Wash the potato eyes in clean water as soon as they are cut. Dusting the eye sets with limestone or magnesium limestone has given good results some years, but has not been as reliable as washing the sets with water.

(5) Package eye sets, as soon as they are washed, in a moisture proof type container. A wax coated carton, wrapped with waxed kraft paper is a reliable package for keeping eye sets in good condition.

(6) Store the eye sets at about 77° F. from four days to a week, and then at 38° F. until shipped. The next best storage is 38° F. continuously. Storages around 60° F. should be avoided.

ACKNOWLEDGMENTS

Acknowledgment is made for the assistance given in field plot technique and statistical analysis by Dr. W. C. Broadfoot and Dr. A. McCalla; and also the co-operation, without which the experiments would not have been possible, of the Field Crops Department of the University of Alberta, who loaned an acre of land each year and assisted in the cultivation and harvesting of the crop.

REFERENCES

1. Bushnell, J. Rate of planting potatoes with references to sprouting habit and size of plants. Ohio Agricultural Experimental Station, Bulletin 462. 1930.

2. Scannell, J. W. Development of the certified seed potato eye trade. American Potato Journal, 14 : 1. January, 1936.

CROWN ROT OF APPLE TREES IN BRITISH COLUMBIA—ROOTSTOCK AND SCION RESISTANCE TRIALS[1]

RANDAL E. FITZPATRICK[2], FRANCES C. MELLOR[3], AND MAURICE F. WELSH[4]

Dominion Laboratory of Plant Pathology, Summerland, B.C.

[Received for publication June 10, 1944]

The trials reported herein are the first of a series that is being made at the request of the Experimental Farms Service as an adjunct to the work of evaluating the suitability of various apple stocks for British Columbia conditions. Since the root crown rot that is caused by *Phytophthora cactorum* is a most prevalent disease in the largest fruit growing region of this province, the Okanagan Valley, resistance to it is a very desirable character. That various stocks might be expected to display differences in susceptibility and resistance was indicated by results obtained in earlier work by Welsh (2) and by the work of Baines (1) on the apple tree trunk canker, the collar rot of Indiana, which is also caused by a strain of *P. cactorum*.

In addition to the resistance trials proper, an experiment to determine whether depth of planting had an effect on the incidence of crown rot was made. As the varieties used in this latter experiment were for the most part the same as those on trial for resistance, the results of this experiment are also included in this report.

The material used was selected, propagated, planted, and cared for by the Experimental Farms Service. For this, and for the design of the field plans, particular credit is due Messrs. A. J. Mann and R. C. Palmer of the Summerland Experimental Station, B.C. In all 1,560 trees, in which were represented 40 different varieties and rootstocks, were planted.

MATERIAL AND METHODS

Planting Plan

Resistance Trials Experiment

The trees were planted 3 feet apart in rows 6 feet apart. There were 24 rows of 52 trees each. Each root-scion combination was originally represented by 16 trees, but in some cases not all survived to the time of testing; casualties were particularly high in the "own-rooted" trees. To compensate for soil variation, the 24 rows were divided into 4 contiguous blocks of 6 rows each. The 16 trees of each combination were split up into groups of 4 and each group placed in a different position in the corresponding rows of the 4 blocks. The arrangement was such that the position of the groups relative to the ends of the rows was different in each block, although the order of the combinations in the rows was the same.

The trees were planted in the spring of 1941, and were then 1 year old.

[1] Contribution No. 786 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Assistant Plant Pathologist.

[3] Agricultural Assistant.

[4] Agricultural Assistant. At present on Active Service, C.A.O.

Depth of Planting Experiment

The depth of planting experiment was conducted in a block similar in size and contiguous to those of the resistance trials. In this block, however, the rows were identical, both in composition and order of planting. Each root-scion combination was represented by 12 trees, 2 in each of the 6 rows. Three depths of planting were used; the first and fourth row with the root-scion union 4 inches below the ground line, the third and sixth with the unions at the ground line, and the second and fifth with the unions 4 inches above it.

Inoculation

A mixture of four isolates[5] of *P. cactorum*, derived from widely separated crown rotted trees, was used as inoculum. The isolates were cultured separately on a mixture of corn meal, sand, and pea extract, and the cultures mixed together just before use.

The inoculations were made by cutting out vertical strips of bark approximately 3/16 inches wide and smearing the inoculum on the cambium thus exposed. No attempt was made to standardize the vertical length of the cut, as this naturally varied with the character of the individual tree, but care was taken to confine the cut to either root or scion, or both, as the test required. Only in those trees in the depth of planting experiment was the inoculation made directly across the union. In the case of the scion tests, the soil was mounded around the base of the trees so that about 3 inches of scion was covered and the wound so protected from immediate drying out.

The inoculations were made during May and June in 1942 on the north sides of the trees. Those trees that had developed no rot by the spring of 1943 were reinoculated during June and July in 1943, this time on the south side.

RESULTS

Resistance Trials Experiment

These trials terminated in the fall of 1943. Records of the extent and position of rot were taken in October. These are presented in Table 1 for the rootstocks and in Table 2 for the scions. In the tables will be found the varietal composition and number of trees of each combination, the percentage that developed crown rot, and an arbitrary disease rating.

The method of arriving at the arbitrary disease rating was as follows: When the records were taken the amount of rot present on each tree was evaluated and noted: no rot, 0; a trace of rot (but less than one-quarter girdling), 0.5; about one-quarter girdling, 1; about one-half girdling, 2; about three-quarters girdling, 3; complete girdling, 4. The disease rating for each combination of root and scion was derived by expressing the sum of the figures so obtained as a percentage of the total possible for that combination had all of the trees been completely girdled. For example, in the combination Malling II root and Red Jonathan scion there were 16 trees (Table 1). Of these, 2 showed a trace of rot in the root or a total of 1. The total possible for 16 trees is 64. Therefore, the disease rating for the

[5] These were:—No. 229 (Summerland, B.C.).
No. 452 (Osoyoos, B.C.).
No. 558 (Summerland, B.C.).
No. 594 (Vernon, B.C.)

TABLE 1.—PATHOGENICITY OF *Phytophthora cactorum* ON VARIOUS APPLE ROOTS ARTIFICIALLY INOCULATED FOR TWO SUCCESSIVE YEARS WITH A MIXED CULTURE OF FOUR ISOLATES

Roots inoculated	Varietal composition of tree		Roots developing crown rot	Disease rating
	Root	Scion		
number	*Seedling roots*		%	
16	Anis	Antonovka	0	0
16		Hibernal	25	20
16		Pyrus baccata	6	6
48			10	9
16	Antonovka	Charlamoff	0	0
16		Hibernal	0	0
16		Virginia crab	0	0
48			0	0
16	Canada Baldwin	Antonovka	44	37
16		Charlamoff	50	37
14		Hibernal	36	34
16		Jubilee	25	22
16		Spartan	19	17
10		Stirling	40	31
16		Virginia crab	69	66
104			40	35
16	Columbia	Columbia	6	6
16		Virginia crab	12	11
32			9	8
16	Duchess	Antonovka	19	8
16		Charlamoff	44	30
16		Hibernal	31	25
16		Virginia crab	69	61
64			41	31
16	Martha	Beauty	19	16
16		Haas	6	6
16		Tony	19	14
16		Virginia crab	19	19
64			16	14
15	McIntosh	Stirling	40	30
15	Pyrus robusta	Pyrus baccata	7	5
16	Wealthy	Hibernal	6	5
15	Yellow Transparent	Hibernal	33	22
16		Charlamoff	63	49
16		Virginia crab	81	67
47			60	46

TABLE 1.—PATHOGENICITY OF *Phytophthora cactorum* ON VARIOUS APPLE ROOTS ARTIFICIALLY INOCULATED FOR TWO SUCCESSIVE YEARS WITH A MIXED CULTURE OF FOUR ISOLATES—*concluded*

Roots inoculated	Varietal composition of tree		Roots developing crown rot	Disease rating
	Root	Scion		
number			%	
	Malling roots			
16	Malling I	McIntosh	37	22
16		Newtown	37	26
16		Red Winesap	50	27
16		Turner Red	88	61
64			53	34
16	Malling II	McIntosh	0	0
16		Newtown	0	0
16		Red Jonathan	12	2
16		Red Winesap	19	8
16		Turner Red	6	6
80			7	3
16	Malling IX	McIntosh	6	6
16	Malling XVI	McIntosh	25	18
	Own roots			
15	Anis		20	20
15	Antonovka		0	0
13	Canada Baldwin		77	63
14	Charlamoff		21	11
2	Delicious		0	0
15	Hibernal		13	13
14	Jubilee		0	0
14	McIntosh		0	0
10	Melba		0	0
16	Virginia crab		0	0
11	Wealthy		9	2
10	160 (stock number)		10	10

Malling II root is $(1 \div 64) \times 100 = 1.56$, or 2 expressed to the nearest whole number. In the Columbia root and Virginia crab scion combination there were likewise 16 trees. Of these, 2 also showed rot, but here one was three-quarters and the other completely girdled giving a total of 7. Therefore, the disease rating for the Columbia root is $(7 \div 64) \times 100 = 11$. It will be noted that in both cases the percentage of roots developing crown rot is the same, namely, 12%.

As the number of trees used in each individual combination was relatively small, neither the figure for percentage of trees developing rot nor that for disease rating can be considered anything more than indicative when the combinations are considered separately. However, some of the varieties were represented in more than one combination and when these are treated as a group the figures become more significant. Thus, in Table 1, Anis, Antonovka, Canada Baldwin, Columbia, Duchess, Martha, Malling I, and Malling II, can be so treated and for each a total percentage and disease rating figure has been calculated.

TABLE 2.—PATHOGENICITY OF *Phytophthora cactorum* ON VARIOUS APPLE SCIONS ARTIFICIALLY INOCULATED FOR TWO SUCCESSIVE YEARS WITH A MIXED CULTURE OF FOUR ISOLATES

Scions inoculated	Varietal composition of tree		Scions developing rot	Disease rating
	Seedling root	Scion		
number			%	
16	McIntosh	Antonovka	0	0
16	McIntosh	Bedford	37	12
16	McIntosh	Canada Baldwin	44	33
16	McIntosh	Charlamoff	6	3
16	McIntosh	Delicious	0	0
16	McIntosh	Dolgo	0	0
16	McIntosh	Florence	6	2
16	McIntosh	Grimes Golden	37	32
16	McIntosh	Haas	0	0
16	McIntosh	Haralson	0	0
16	McIntosh	Hibernal	19	17
16	McIntosh	Hyslop	44	41
14	McIntosh	Jubilee	7	7
12	McIntosh	McIntosh	0	0
16	Canada Baldwin	Newtown	0	0
16	McIntosh	Olga	69	61
16	McIntosh	Osman	12	12
28	McIntosh	Pyrus baccata	39	27
16	McIntosh	Robin	0	0
15	McIntosh	Spartan	0	0
16	McIntosh	Tony	6	3
16	McIntosh	Transcendent	0	0
16	McIntosh	Virginia crab	6	5
16	McIntosh	Winesap	12	8
16	McIntosh	Winter St. Lawrence	81	81
4	McIntosh	Wolf River	75	75

Depth of Planting Experiment

The records for the depth of planting experiment were taken at the same time as were those for the resistance trials and are summarized in Table 3. In the trees planted with the union at the ground line, slightly more rot developed in the roots than in those planted deeper or shallower, and deep planting was more conducive to rot in the scion than was ground line or shallow planting. However, there was little significant difference in the total amounts of rot which developed on the three groups.

TABLE 3.—EFFECT OF DEPTH OF PLANTING ON INCIDENCE OF CROWN ROT*

Depth of planting	Rot in roots		Rot in scions		Total rot in trees	
	Affected trees	Disease rating	Affected trees	Disease rating	Affected trees	Disease rating
	%		%		%	
Union 4″ below ground line	20	16	25	20	34	28
Union at ground line	31	22	17	13	38	28
Union 4″ above ground line	22	15	18	12	33	21

There were 104 for each depth; 52 combinations represented; inoculated for 2 successive years with a mixed culture of 4 isolates, *Phytophthora cactorum;* inoculation across union.

TABLE 4.—PATHOGENICITY OF *Phytophthora cactorum* ON VARIOUS APPLE ROOTS AND SCIONS BASED ON THE RESULTS OF THE DEPTH OF PLANTING EXPERIMENT*

Crown rot in root		Varietal composition of tree		Crown rot in scion	
Roots developing crown rot	Disease rating	Seedling root	Scion	Scions developing crown rot	Disease rating
%				%	
8	8	Antonovka	Charlamoff	17	9
8	1		Virginia crab	0	0
8	5				
42	27	Canada Baldwin	Antonovka	0	0
33	29		Charlamoff	33	26
38	28				
0	0	Columbia	Columbia	8	1
25	17	Duchess	Antonovka	0	0
75	45		Charlamoff	25	15
75	61		Virginia crab	33	22
58	41				
33	25	Martha	Beauty	58	50
0	0		Haas	0	0
17	8		Tony	8	6
25	11				
42	25	McIntosh	Bedford	17	10
8	8		Canada Baldwin	17	17
17	15		Dolgo	0	0
25	14		Haas	0	0
17	10		Hyslop	50	36
17	12		Olga	42	38
8	8		Osman	0	0
8	4		Pyrus baccata	25	17
0	0		Robin	0	0
33	25		Tony	25	19
17	12		Transcendent	8	4
42	27		Winter St. Lawrence	92	81
20	13				
8	8	Pyrus robusta	Pyrus baccata	33	25
42	28	Yellow Transparent	Antonovka	0	0
42	35		Charlamoff	33	25
42	32				

* Inoculation across the union for 2 successive years with a mixed culture of 4 isolates; each combination represented by 12 trees.

Since the combinations used in the depth of planting were, for the most part, ones which were also used in the resistance trials experiment, and since depth of planting had so little influence on the incidence of crown rot, the results in the individual combinations used here may be compared with those obtained from the resistance trials. Table 4 gives the stock-scion combinations used and the percentage and disease ratings for each.

DISCUSSION OF RESULTS

It must be emphasized again that the results obtained from these trials can be considered to be indicative only. This is particularly true for the results on individual combinations and where the material was represented only once in the various combinations, as this meant a test on 16 trees or less. On the other hand, much of the material was represented in several combinations in the resistance trials experiment or occurred both in this and in the depth of planting experiment; moreover, the maternal parents of some of the seedling roots were used on their own roots or as scions on other rootstocks. By combining all of this information a very fair estimate of the comparative resistance and susceptibility of much of the material can be made. Table 5 which lists the disease ratings as obtained from these various sources may be used for this purpose.

It will be seen from Table 5 and the preceding tables that certain varieties and their seedlings showed a consistently higher degree of susceptibility than did others. Eleven: Beauty, Canada Baldwin, Duchess, Hyslop, Grimes Golden, Malling I, Olga, Winter St. Lawrence, Yellow Transparent, Wolf River, and *Pyrus baccata*, have all proved to be highly susceptible. Eleven: Anis, Bedford, Charlamoff, Hibernal, Malling XVI, Martha, McIntosh, Osman, Stock 160, Tony and Virginia crab, were at least partially susceptible. Seven: Antonovka, Columbia, Dolgo, Haas, Malling II, Robin and Transcendent were quite resistant. Eleven: Delicious, Florence, Haralson, Jubilee, Malling IX, Melba, Newtown, *Pyrus robusta*, Spartan, Wealthy, and Winesap, although showing good resistance, must still be considered to be in doubt because of inadequate numbers and lack of cross checks.

While the evaluation of resistance and susceptibility which was obtained from these trials is probably substantially correct, the possibility that one partner of the root-scion combination may have a modifying effect on the resistance of the other, should not be overlooked. Some evidence that such an effect exists may be seen in the behaviour of Virginia crab on the various rootstocks with which it was combined. This variety was found to be relatively resistant. The 16 own-rooted trees showed no crown rot, and when used as a scion on the resistant Antonovka root, only a trace of rot developed in 1 tree out of 28 inoculated. On the other hand, when Virginia crab was used on the susceptible seedling roots of Canada Baldwin, Duchess, and Yellow Transparent, the incidence of crown rot in these seedling roots was far greater than when these seedlings were combined with any other variety, in some cases the disease rating being double the average rating for these roots.

SUMMARY

The susceptibility of 40 different apple varieties and seedlings to the crown rot caused by *P. cactorum* in British Columbia was compared by artificially inoculating 1,560 trees.

TABLE 5.—CROWN ROT DISEASE RATINGS (TOGETHER WITH NUMBERS OF TREES ON WHICH THESE RATINGS ARE BASED) OF VARIOUS APPLE VARIETIES AND SEEDLINGS AS OBTAINED IN THE DIFFERENT EXPERIMENTS

Material	Seedling		Variety		
	Resistance trials	Depth of planting	Own-rooted	Scion on other roots	
				Trials	Depth of planting
Anis	9 (48)	— —	20 (15)	— —	— —
Antonovka	0 (48)	5 (24)	0 (15)	0 (16)	0 (36)
Beauty	— —	— —	— —	— —	50 (12)
Bedford	— —	— —	— —	12 (16)	10 (12)
Canada Baldwin	35 (104)	28 (24)	63 (13)	33 (16)	17 (12)
Charlamoff	— —	— —	11 (14)	3 (16)	19 (48)
Columbia	8 (32)	0 (12)	— —	— —	1 (12)
Delicious	— —	— —	0 (2)	0 (16)	— —
Dolgo	— —	— —	— —	0 (16)	0 (12)
Duchess	31 (64)	41 (36)	— —	— —	— —
Florence	— —	— —	— —	2 (16)	— —
Grimes Golden	— —	— —	— —	32 (16)	— —
Haas	— —	— —	— —	0 (16)	0 (24)
Haralson	— —	— —	— —	0 (16)	— —
Hibernal	— —	— —	13 (15)	17 (16)	— —
Hyslop	— —	— —	— —	41 (16)	36 (12)
Jubilee	— —	— —	0 (14)	7 (14)	— —
Malling I	— —	— —	34* (64)	— —	— —
Malling II	— —	— —	3* (80)	— —	— —
Malling IX	— —	— —	6* (16)	— —	— —
Malling XVI	— —	— —	18* (16)	— —	— —
Martha	14 (64)	11 (36)	— —	— —	— —
McIntosh	30 (15)	13 (144)	0 (14)	0 (12)	— —
Melba	— —	— —	0 (10)	— —	— —
Newtown	— —	— —	— —	0 (16)	— —
Olga	— —	— —	— —	61 (16)	38 (12)
Osman	— —	— —	— —	12 (16)	— —
Pyrus baccata	— —	— —	— —	27 (28)	21 (24)
Pyrus robusta	5 (15)	8 (12)	— —	— —	— —
Robin	— —	— —	— —	0 (16)	0 (12)
Spartan	— —	— —	— —	0 (15)	— —
Tony	— —	— —	— —	3 (16)	13 (24)
Transcendent	— —	— —	— —	0 (16)	4 (12)
Virginia crab	— —	— —	0 (16)	5 (15)	11 (24)
Wealthy	5 (16)	— —	2 (11)	— —	— —
Winesap	— —	— —	— —	8 (16)	— —
Winter St. Lawrence	— —	— —	— —	81 (16)	81 (12)
Wolf River	— —	— —	— —	75 (4)	— —
Yellow Transparent	46 (47)	32 (24)	— —	— —	— —
160 (stock number)	— —	— —	10 (10)	— —	— —

* Roots budded to various scions.

Of those tested, 11 proved to be quite susceptible, 11 partially so, and 7 were resistant, while the data obtained on the remaining 11 were deemed insufficient to warrant an evaluation.

In an experiment on the effect of depth of planting on incidence of crown rot there was no significant difference in the amount of rot that developed when the trees were planted with the union 4 inches above the ground line, at the ground line, and 4 inches below it.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Messrs. A. J. Mann and R. C. Palmer of the Summerland Experimental Station for their generosity, both in material and time, that in no small measure contributed to the success of the project, and to Dr. H. R. McLarty of the Dominion Laboratory of Plant Pathology, Summerland, for his aid and advice.

REFERENCES

1. Baines, R. C. Phytophthora trunk canker or collar rot of apple trees. J. A. R. 59 : 159-184. 1939.

2. Welsh, M. F. Studies of crown rot of apple trees. Can. Jour. Res. 20 : 457-490. 1942.

RESISTANCE OF APPLE SEEDLINGS TO SCAB[1]

J. E. Hockey[2] and C. C. Eidt[3]

[Received for publication May 13, 1944]

The orchardists in Nova Scotia, as in many parts of the continent, suffer partial crop losses annually from the fungus disease known as apple scab (*Venturia inaequalis*). Varieties of apples now in production have been selected for their hardiness, quality, and yield, but little apparent progress has been made in obtaining varieties resistant to scab.

Many of the leading commercial varieties, as McIntosh, Delicious, Gravenstein, Wagener, Baldwin, and Stark are susceptible to scab and require a consistent fungicide treatment to protect them from this disease. Varieties such as Golden Russet, Ribston, and Cox Orange Pippin are less susceptible. Some resistance has been shown by the varieties Blenheim, Orange Pippin, and Yellow Transparent but the latter variety is of little use as a fruit for export. Among the varieties grown at the Dominion Experimental Station, Kentville, N.S., and observed as resistant to scab are Red Winter Reinette and Belle de Boskoop. These two apples differ considerably. The Boskoop resembles the Blenheim in colour and season but is poorer in quality, and, being a triploid, is an unsatisfactory parent. The Red Winter Reinette is a smaller apple, well coloured, a good keeper, and has fair quality; being a diploid, it is a useful parent in a breeding program.

During the past 10 years a collection of approximately 30,000 apple seedlings of known varietal parentage has been accumulated at the Station under the direction of the junior author. These have been planted in nurseries and allowed to develop for 5 or 6 years without fungicide protection. Observations have been made on growth, vigour, disease resistance, etc., of these seedlings. In their early life the foliage reaction to scab has been the main disease observation. Some records have been made on the reaction of the fruit to scab but the mass of seedlings are not yet in production.

It is the purpose of this paper to present a summary of the observations on foliage scab of those seedlings which have been under observation for at least four years, during which time the disease was quite prevalent. No attempt at artificial inoculation was made. The data secured are based entirely on natural infection by the fungus in the nurseries. Studies on the strains of the fungus present and the effect of the resistant host on the fungus are not dealt with in this paper.

[1] Contribution No. 780, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada, and Contribution No. 565 from the Division of Horticulture, Experimental Farms Service, Department of Agriculture, Ottawa.

[2] Pathologist-in-Charge, Dominion Laboratory of Plant Pathology, Science Service, Dominion Department of Agriculture, Kentville, N.S.

[3] Assistant in Plant Breeding, Dominion Experimental Station, Experimental Farm Service, Dominion Department of Agriculture, Kentville, N.S.

MATERIALS AND METHODS

Source of Seedlings

The seedlings were obtained from two sources:

(a) Known crosses were made using varieties with desirable characters. The trees were tented in the "pink stage" and immediately before bloom all buds were removed except two lateral buds in each cluster that were emasculated. The pollen for the crosses was secured by removing anthers from unopened buds, and maturing in sunlight. Pollination was performed by dipping the receptive stigmas into a metal box containing the pollen. Individual branches were used for each cross. The checks emasculated that were not pollinated set no fruit.

(b) The second source of material was from pollination studies where emasculation was not practised. A limb unit method was used and 10 replications were made of each cross on separate trees. At the late pink stage of the bloom each limb was bagged with cheese cloth to prevent cross pollination. At the full bloom period the centre flowers were removed, and those remaining in the cluster were hand pollinated. The pollen was applied to the stigmas with a camel's hair brush and alcohol was used to sterilize the brushes after each cross. It is recognized that some selfing may occur when this method is used. However, varieties which were self-pollinated gave such low sets of fruit when compared with compatible crosses that it is believed few of the selfed flowers developed seeds.

General Character of Diploid and Triploid Crosses

Reciprocal crosses were made using diploid and triploid varieties. In addition these have been selfed. A summary of the fruit set, seed germination, and seedling growth of the majority of crosses, grouped according to the chromosome number of the parents, is given in Table 1.

The percentage of fruit set was much higher in the diploid × diploid, and in the triploid × diploid crosses than in the other groups. The relatively high set of 3.7% for selfed triploids is explained by the self-fruitfulness of the varieties Baldwin and Rhode Island Greening which were included in this summary. It is interesting to note that all diploid crosses had a higher seed content per fruit than those where triploids were the female parents.

Seed germination records were taken each year. The results for 1933 only are given in Table 1. The seed from diploid × diploid and diploid selfed groups has consistently germinated better than that from triploid crosses.

Seedlings were grown for one year in the seed bed and then transplanted to a permanent location. In the seed bed it was apparent that seedlings of the diploid × diploid crosses or diploid selfed were more vigorous and uniform than the seedlings of the other groups. Seedlings from the triploid crosses usually lacked vigour, very few have grown as well as many of the diploid seedlings. These few vigorous plants from the triploid crosses maintained their vigour in permanent location; no determinations have been made of their chromosome numbers.

Many of the seedlings from triploid crosses winter killed or died soon after permanent planting. After 2 years the average height of the triploid

TABLE 1.—SET, GERMINATION, AND GROWTH OF SEED AND SEEDLINGS

Type of cross	Percentage fruit set	Average no. seeds per apple	Germination*	Stand†	Height of seedlings†
	%		%	%	Feet
Diploid × diploid	14.60	7.6	82.3	97.6	5
Diploid × triploid	2.00	5.9	28.7	26.4	4
Triploid × diploid	17.50	3.4	22.4	18.7	2
Triploid × triploid	2.90	3.2	2.6	34.7	2
Diploid self	1.70	4.4	64.9	92.1	5
Triploid self	3.70	3.1	17.8	31.2	—

* 1933 Results.

† Records taken after 2 years in permanent location. Plants set out as 1 year seedlings.

crosses was less than that of the diploids. Since seed content and germination of the triploid crosses were reduced, and since the stand and the vigour of the resulting triploid seedlings were also reduced these crosses have produced few plants for the determination of scab resistance. From the plant breeder's standpoint triploid varieties of apples have proved unsatisfactory as male and female parents.

In addition to the variations in seedling vigour mentioned above there has been an appreciable difference in growth of the plants due to the presence of scab. Seedlings from resistant parents, as Red Winter Reinette, produced an average of 18 inches growth the first year. Many seedlings from susceptible parents, as McIntosh and Delicious, were unable to make more than 4 to 6 inches growth in the same period due to severe infestations of scab on the foliage. This effect continued in the permanent planting until fungicide sprays were used.

Where a cross had been made between a resistant and a susceptible variety, the resistant variety had an effect on the resistance of the progeny. For example, in the Red Winter Reinette × McIntosh cross, although scab susceptible individuals have appeared, in no case has the scab been severe enough to defoliate the trees or stunt seedling growth.

No estimate of hereditary vigour has been possible, due to the dwarfing of the seedlings by scab.

Methods of Disease Observation

The observations on foliage scab have been made during July and August of each year. The system used in recording the disease was as follows:

1. No scab present.
2. Scab spots isolated or distinctly separate.
3. and 4. (Not used in this classification.)
5. Scab very diffuse, covering large areas.
6. Scab spots killed out; brown spots or flecks.

Following each number or combination of numbers denoting the presence of scab was a letter indicating the percentage ranges:

A—less than 10% of the foliage affected.

B—10 to 50% of the foliage affected.
C—more than 50% of the foliage affected.
A numerical system was also used to record scab on the fruit as follows:
0—No scab.
1—Few small spots, pronounced cork layer.
2—(Not used in this classification.)
3—Small to medium scattered spots.
4—Spots one-half inch or more in diameter; no normal cracking of fruit.
5—Large active spots, coalescing; fruit cracking.

These methods of classification have been found quite satisfactory for rating the scab reactions of apple varieties and seedling trees. By giving each rating a numerical value, it has been possible to get an average rating for the period under observation and to reduce the seedlings into the four groups:

Susceptible (S), slightly susceptible (SS), slightly resistant (SR) and resistant (R) as given in Tables 2 and 3.

Varietal susceptibility of Seedling Parents

Observations on the scab susceptibility of standard varieties were based largely on fruit lesions as it has not been economically practicable to allow a variety orchard to go without fungicide protection. A summary of the scab observations on the standard varieties represented as parents among the seedlings reported in this paper is given in Table 2. It will be noticed that the preponderance of commercial varieties is in the two susceptible groups.

TABLE 2.—REACTION TO SCAB OF SEEDLING PARENTS

Susceptible	Slightly susceptible	Slightly resistant	Resistant
Baldwin	Beauty of Bath	Adams Pearmain	Red Winter Reinette
Gravenstein*	Ben Davis	Cox Orange	
Lobo	Crimson Beauty	Deacon Jones	
McIntosh	Evangeline	Early Williams	
Red Delicious	Golden Delicious	English Russet	
Rome Beauty	Hubbardson	Golden Russet	
Wagener	Jonathan	Grimes Golden	
	Northern Spy	Mother	
	Red Astrachan	Opalescent	
	Tompkins King*	Yellow Transparent	
	Worcester Pearmain	York Imperial	
	Yellow Bellflower		

* Triploid varieties.

Foliage Scab of Seedlings

Rather than enter into a detailed discussion of the individual crosses and their reaction to foliage scab, the seedlings are classified in Table 3, according to susceptibility. This classification is limited to those crosses in which there were 50 or more seedlings. The figures in parentheses following the variety name (Table 3) indicate the number of years in which this cross was made. All replicate crosses were totalled and the figures represent the percentage of seedlings in each of the 4 classes. The right-hand column gives the total number of seedlings in each cross.

TABLE 3—SEEDLING SUSCEPTIBILITY BY CROSSES

——	S	SS	SR	R	Total
Baldwin					
× Cox Orange (4)	9.9	26.7	42.5	20.8	101
× Northern Spy (3)	14.3	15.8	47.6	22.2	63
Ben Davis					
× Adams Pearmain	70.3	26.6	3.1	0.0	64
× Cox Orange	72.0	18.6	9.3	0.0	75
× Crimson Beauty	47.7	38.9	13.3	0.0	113
× Deacon Jones (2)	36.9	33.7	29.3	0.0	160
× Delicious (2)	72.6	21.6	5.8	0.0	51
× Golden Russet	54.5	27.2	16.7	1.5	132
× Opalescent	78.4	19.8	1.8	0.0	111
× Red Astrachan	60.9	33.3	4.3	1.4	69
× Red Spy	56.8	34.9	7.2	0.0	83
× Wagener	81.1	14.5	4.3	0.0	69
× Yellow Bellflower	39.3	45.9	14.7	0.0	61
Cox Orange					
× Baldwin (3)	28.5	42.8	28.5	0.0	70
× Deacon Jones (2)	44.0	52.0	4.0	0.0	50
× Delicious	43.7	45.4	10.9	0.0	320
× Early Williams	43.2	42.4	14.4	0.0	132
× Golden Russet (4)	38.8	36.8	22.3	2.1	1187
× Gravenstein (3)	33.0	44.8	21.2	0.9	203
× King (3)	32.0	34.7	32.0	1.3	75
× Lobo	38.6	40.5	19.6	1.2	158
× McIntosh (4)	51.8	35.8	12.2	0.1	664
× Red Astrachan	24.0	56.0	20.0	0.0	50
× Red Spy	33.8	44.1	19.1	2.9	68
× Red Rome Beauty	49.2	30.7	18.5	1.5	65
× Wagener (4)	52.7	42.3	4.8	0.1	690
× York Imperial	34.7	41.1	23.0	1.1	265
× Self (3)	37.2	32.1	28.1	2.5	199
Open pollinated	42.3	28.5	27.7	1.4	137
Deacon Jones					
× Beauty of Bath	52.4	29.5	14.7	3.3	61
× Crimson Beauty	40.4	42.2	17.4	0.0	109
× Golden Delicious	73.0	23.8	3.1	0.0	63
× Wagener	76.7	23.2	0.0	0.0	56
× Yellow Transparent (2)	12.8	47.7	39.4	0.0	109
× York Imperial	62.5	20.2	6.7	10.5	104
Open pollinated (2)	47.0	39.9	12.9	0.2	394
Delicious					
× Ben Davis	88.8	11.1	0.0	0.0	54
× Deacon Jones	65.3	28.5	6.1	0.0	98
× Northern Spy	91.4	6.9	1.7	0.0	58
× Yellow Transparent	55.9	39.3	4.7	0.0	84
Open pollinated	71.9	22.5	4.5	1.1	89
Golden Russet					
× Baldwin (2)	33.9	29.0	35.4	1.6	62
× Cox Orange (3)	57.3	31.3	10.8	0.5	543
× Gravenstein (3)	15.1	50.0	33.3	1.5	66
× McIntosh (3)	42.5	36.1	20.1	1.2	342
Gravenstein					
× Wagener (4)	4.7	14.1	57.7	23.5	85
McIntosh					
× Ben Davis	91.4	8.5	0.0	0.0	70
× Cox Orange (2)	38.3	43.2	18.5	0.0	81

TABLE 3.—SEEDLING SUSCEPTIBILITY BY CROSSES—*Continued*

——	S	SS	SR	R	Total
McIntosh—Concluded					
× Deacon Jones	63.8	30.3	5.8	0.0	221
× Delicious (2)	81.7	17.3	1.0	0.0	98
× English Russet	87.2	11.3	1.4	0.0	71
× Golden Russet	81.0	17.4	1.5	0.0	63
× Hubbardson	46.0	32.4	20.2	1.3	74
× Jonathan	75.5	22.6	1.8	0.0	53
× Opalescent (2)	75.3	24.6	0.0	0.0	73
× Red Spy	91.3	8.7	0.0	0.0	104
× Red Winter Reinette	22.8	52.4	24.2	0.5	360
× Worcester Pearmain (2)	19.7	56.0	24.2	0.0	66
Open pollinated	46.5	40.7	12.7	0.0	535
Mother					
× Ben Davis	59.1	30.7	10.1	0.0	218
× Crimson Beauty	33.3	50.8	15.8	0.0	63
× Deacon Jones (2)	35.1	41.4	21.6	1.8	111
× Delicious	68.3	22.2	8.3	1.1	180
× Early Williams	57.5	29.9	11.5	1.1	87
× Golden Russet	41.1	39.3	19.6	0.0	56
× Grimes Golden	66.7	19.0	12.7	1.5	63
× Lobo	75.7	17.0	6.8	0.6	147
× Opalescent	58.7	31.2	9.5	0.5	189
× Red Astrachan	74.0	23.9	2.1	0.0	142
× Red Rome Beauty (2)	46.1	39.0	14.9	0.0	141
× Worcester Pearmain	18.0	54.0	28.0	0.0	50
× Yellow Bellflower	60.2	30.1	7.5	2.1	93
× Yellow Transparent	15.9	42.6	38.3	3.1	94
× York Imperial	35.4	43.3	19.7	1.5	127
Opalescent					
× Yellow Transparent	27.7	34.7	37.5	0.0	72
Red Rome Beauty					
× Ben Davis (2)	83.7	15.2	1.1	0.0	92
× Deacon Jones (2)	50.4	29.6	19.2	0.8	125
× Delicious (2)	77.9	20.6	1.4	0.0	68
× Early Russet	85.7	14.3	0.0	0.0	70
× Red Spy	84.1	15.9	0.0	0.0	63
Open pollinated	56.9	32.7	10.0	0.3	309
Red Winter Reinette					
× Ben Davis	25.5	47.0	25.5	1.9	153
× Cox Orange (2)	22.4	37.1	40.5	0.0	116
× Delicious	21.7	37.2	40.7	0.3	258
× Deacon Jones	28.7	42.1	28.1	1.1	171
× Jonathan	25.4	60.1	14.5	0.0	55
× McIntosh	43.4	40.2	16.4	0.0	279
× Opalescent (2)	32.0	45.8	21.8	0.3	275
× Red Astrachan	28.5	45.4	24.6	1.3	77
× Red Rome Beauty	33.7	48.9	17.4	0.0	92
× Red Spy	20.6	46.8	31.7	0.8	126
× Wagener	68.0	28.2	3.8	0.0	78
× Yellow Transparent	5.4	32.7	54.5	7.3	75
Open pollinated	36.4	36.6	26.1	0.9	1059
Spy, Northern					
× Baldwin (3)	36.2	51.7	10.3	1.7	58
× Ben Davis (3)	62.9	29.4	7.3	0.3	1559
× Cox Orange (3)	34.9	37.1	26.9	1.1	1998
× Golden Russet (3)	54.9	31.9	13.1	0.1	856
× King (3)	35.4	40.2	21.9	2.4	82
× Self (2)	50.8	33.3	12.3	3.5	57

TABLE 3.—SEEDLING SUSCEPTIBILITY BY CROSSES—*Concluded*

——	S	SS	SR	R	Total
Spy, Red					
× Deacon Jones (2)	38.9	45.5	15.5	0.0	77
× McIntosh	60.2	33.3	5.3	1.1	93
× Red Rome Beauty (2)	72.9	23.6	3.4	0.0	144
× Red Winter Reinette	29.2	50.6	20.2	0.0	89
Wealthy					
× Deacon Jones	22.0	44.1	33.8	0.0	68
Yellow Bellflower					
× Red Winter Reinette	17.1	52.8	30.0	0.0	70

Table 3 presents many interesting comparisons on the behaviour of varieties as male or female parents. Previous mention has been made of the behaviour of triploids when used as female parents and the small number of seedlings obtained from such crosses. The varieties Baldwin and Gravenstein, when used as female parents for several successive years were the only triploids which produced a sufficient number of seedlings to enable their inclusion in this table. The triploid variety King is represented only as a male parent in Table 3. All other varieties in this table are believed to be diploids.

The apparent resistance of the Baldwin and Gravenstein seedlings requires some explanation. Reference to the height of seedlings, Table 1, shows that the triploid seedlings were less than half the height of diploids. These seedlings lacked vigour. Their leaves were small and frequently late in developing. Their apparent resistance to scab was probably due to avoiding infection because of lateness of spring growth and scarcity of foliage. Many of these seedlings were removed after the fourth year of scab observation on account of weak or undesirable growth characters. A few triploid seedlings have been retained in the planting for fruiting characters but the majority of these fall in the susceptible colum in Table 3. When triploids were used as male parents on diploid varieties the dwarfing effect was not as evident and there was less apparent resistance to scab than was encountered among the seedlings from triploid female parents. Triploid varieties of apples have been very unsatisfactory in this study. The low yield of seed, poor seed germination, and small stand of seedlings have made the triploid varieties of apples of little value for breeding purposes. The inclusion in Table 3 of the few crosses with triploid parents was done to emphasize the difficulties encountered, and point out the undesirability of triploid apple seedlings in scab resistance observations.

Diploid varieties have responded very differently from the triploids. In yield of seed, seed germination, and stand of seedlings they have been most satisfactory. In the seedling stage many were dwarfed temporarily from the effects of scab on the foliage. The seedlings from McIntosh, Delicious, and Rome Beauty were very susceptible to scab in the seed bed. This effect is still shown in Table 3, where it will be observed that approximately 90% of the seedlings of these 3 varieties fall in the susceptible and

slightly susceptible columns. Scab susceptibility appears to be a dominant factor in these varieties.

In contrast to the extremely susceptible varieties are those more resistant. The 3 varieties Red Winter Reinette, Golden Russet, and Cox Orange have over 25% of their seedlings classified in the slightly resistant and resistant columns of Table 3. The percentages of these seedlings susceptible to scab are much lower than those of the seedlings of the 3 susceptible varieties mentioned in the preceding paragraph.

If the data given in Table 3 are placed in 2 columns instead of 4 it will be readily seen that all diploid seedlings with the exception of the Red Winter Reinette × Yellow Transparent cross, have over half the seedlings in the scab susceptible groups. Such a grouping would indicate that resistance to scab is probably a recessive character in the diploid apple varieties used in this project.

With the exception of Delicious × Yellow Transparent, all other crosses with Yellow Transparent as a male parent have given over 37% of the seedlings showing a degree of resistance. The early blooming date of this variety made it difficult to obtain successfully crosses with Yellow Transparent as a female parent. A further study on progeny of this variety from the standpoint of scab susceptibility would appear desirable.

There are a few crosses with their reciprocals represented in Table 3. The slightly susceptible Ben Davis × the susceptible Delicious has given over 94% of all seedlings in the two scab susceptible classes. Practically the same condition is found with McIntosh × Red Spy and Rome Beauty × Red Spy and their reciprocals. In these crosses resistance appears to be recessive. When two slightly resistant parents, Cox Orange and Golden Russet, are crossed a larger proportion of the seedlings fall in the 2 resistant classes. Resistance to scab is present in these varieties but must be considered recessive on the evidence obtained. When the resistant Red Winter Reinette was used in crosses with the susceptible varieties McIntosh and Red Spy a still higher proportion of slightly resistant and resistant seedlings were obtained. Red Winter Reinette appears to carry more resistance than any of the other varieties but the factor for resistance to scab is apparently recessive.

From the data presented in Table 3 it is apparent that susceptibility appears more frequently than resistance, and is a dominant character of many of the best quality varieties. Complete immunity to scab has not been obtained in any of the seedlings from standard varieties. The nearest approach to immunity has been observed locally in seedlings of *Pyrus niedwetzkyana*.

SUMMARY

1. The progeny of over 100 apple crosses has been studied for a period of at least four years and the degree of susceptibility of the seedlings to apple scab (*Venturia inaequalis*) has been recorded.

2. Evidence is presented to indicate that resistance to apple scab appears recessive to susceptibility.

3. Triploid varieties of apples have been unsatisfactory from the breeders standpoint on account of the low seed content, poor germination,

and low vigour of the seedlings. Their apparent resistance is believed to be due to avoiding infection because of delayed foliation rather than true resistance.

4. In the early stages of growth low apparent vigour in scab-susceptible seedlings has been due to severe infections of the disease.

5. The variety Red Winter Reinette has been the most resistant parent used in these crosses. The varieties Cox Orange and Golden Russet have given good proportions of resistant progeny.

6. Mention is made of the possibilities of resistance from crosses using the variety Yellow Transparent.

ACKNOWLEDGMENTS

Acknowledgments are made to Messrs .J. A. Boyle, D. W. Creelman, and R. J. Baylis, who have materially assisted in taking the scab records on the seedlings and to R. J. Hilton for suggestions in the preparation of the manuscript.